OXIDATION BY ATMOSPHERIC OXYGEN

(AUTOXIDATION)

1659

Atmospheric oxygen is the most universally prevalent as well as economically important oxidizing agent for fats and fatty acids. Its action on fats and fatty products may be beneficial or deleterious depending on the conditions and circumstances under which it occurs. It is advantageously employed in the production of blown oils and in various oxidation and polymerization products in the drying oil industry. The process of film formation in applied protective coatings is essentially an oxidative process. The ultimate failure of these same protective coatings, however, is a result of prolonged and excessive oxidation. Rancidification and other forms of deterioration of many fats and fat-containing materials are likewise due to atmospheric oxidation. These reactions are highly undesirable and result in serious economic losses.

So far as we are aware both the favorable and unfavorable results of atmospheric oxidation follow from the same type of reactions between oxygen and the unsaturated constituents of fats or fatty acids. Consequently an enormous amount of effort has been expended in attempting to unravel the mystery which has enshrouded the fundamental reactions involved in these processes. A vast literature has appeared on the subject since about 1830 but it has been only within the past decade that a reasonably clear understanding of the reaction mechanisms has appeared.

Because of the spontaneous nature of the reaction between atmospheric oxygen and unsaturated fats and fatty acids, the process is frequently referred to as autoxidation. Without attempting to justify this terminology, it is used here because of its convenience and because it affords a means

of avoiding the repetitious use of the cumbersome phrase "oxidation with atmospheric oxygen."

Light, heat, concentration of oxygen, moisture, and the presence of catalysts or inhibitors affect the reaction between oxygen and unsaturated fatty acids, often with seemingly very different results. However, it is probable that the same or very similar mechanisms are involved or would ultimately be involved if the reaction process were permitted to run its course. It is sometimes difficult to evaluate the effect of a specific environmental factor in the overall oxidation process because in most cases several of these factors are simultaneously active. Of the several factors which may be operating, one may predominate under one set of conditions and quite a different one under another. For example, in a relatively thick layer of fat or fatty acid maintained in a completely filled glass container at room temperature and exposed to sunlight or ultraviolet light, the absorbed radiant energy may be the predominant factor influencing the oxidation reaction, and the temperature and concentration of oxygen may be quite secondary. On the other hand, in the process of blowing oils the temperature and concentration of oxygen are the predominant factors and the effect of light is secondary.

Catalysts or inhibitors of various types may be added to or may be present naturally in fats and oils, thus markedly influencing the reaction velocity. For example, traces of copper accidentally introduced in a fat or fatty acid increase the rate of oxidative rancidification. The addition of metal oxides or metallic salts in the manufacture of boiled oils accelerates the oxidation and subsequent polymerization reactions in this process, and similar use of these substances in protective coatings enhances their rate of drying and film formation. On the other hand, the presence of antioxidants acts to inhibit

these same reactions.

In planning and executing an investigation in the autoxidation of fats, the careful worker attempts to minimize the number of variables which may affect the course of the reaction, or at least control them within relatively narrow limits. Thus, the effect of light may be avoided by excluding it entirely from the reaction, or the effect of heat may be minimized by maintaining a constant low temperature in the reacting system. Unfortunately the literature contains reports of many investigations in which little or no control was exercised over the various environmental factors, or if they were controlled the author failed to mention it. In such cases it is often difficult to evaluate the data reported or determine the validity of the conclusions drawn by the investigator.

Underlying all investigations of the autoxidation of fats is a desire not only to learn the nature of the products formed but also to understand the mechanisms involved in their production, since only by control of these mechanisms can the desired products be produced or the undesirable products be avoided. Natural fats are generally too complex to permit drawing far-reaching generalizations concerning the mechanisms involved in autoxidative processes. Hence, much work involving these reactions, especially during the past twenty years, has been carried out with simple substances such as oleic, linoleic, or similar acids and their monoesters, because they can be obtained in pure form. Generalizations made on the basis of the results with these simple substances have then been applied to natural fats. In some cases such generalizations may be valid but as in all cases of reasoning by analogy they may not be entirely justified. It should therefore be borne in mind that applying the results and conclusions derived from one substrate, such as methyl oleate at 100°C., to a quite different substrate, such as cottonseed or soybean

oil at room temperature, may not be entirely justified. It is known, for example, that peroxides are relatively unstable at or above 100°C., whereas at room temperature they are relatively stable; therefore quite different end products may be produced under the two reaction conditions. Of course, if the time be sufficiently extended at the lower temperature the same end result might ultimately be achieved although it cannot be assumed that this will be the case. If these limitations are borne in mind, however, it is entirely possible to proceed from the simple to the more complex with reasonable prospects of ultimately resolving the whole of the complicated phenomena involved in the autoxidation of fats.

b. Modern Trends

Prior to about 1940 most investigators studied the autoxidation of the fats themselves. This approach not only introduced many additional and complicating reactions but it made the interpretation of analytical results difficult, if not impossible. In addition, it soon became evident that some analytical methods were not reliable when applied to autoxidation mixtures, thus making some of the early conclusions dubious. Furthermore, before 1940 modern instruments, such as ultraviolet and infrared spectrophotometers and the polarograph, and efficient separation methods, such as urea complexing techniques, countercurrent distribution, chromatography, molecular and fractional distillation, and low temperature crystallization, were either not generally available or were incompletely developed.

Modern investigators not only availed themselves of new instruments and separation techniques but they also studied a large number of highly purified model compounds. Many of these were not derived from fats but contained structures known to be present in fats and in autoxidizing systems. The autoxidation process was studied in detail kinetically and greater insight was obtained into the phenomena of catalysis and inhibition.

The large increase in activity in autoxidation has resulted in a flood of publications. For the student who wishes more detail than can be given in this chapter, some excellent reviews have been published recently on various phases of the autoxidation process.[1-13] (This is only a partial list).

2. Development of the Concepts of Autoxidation

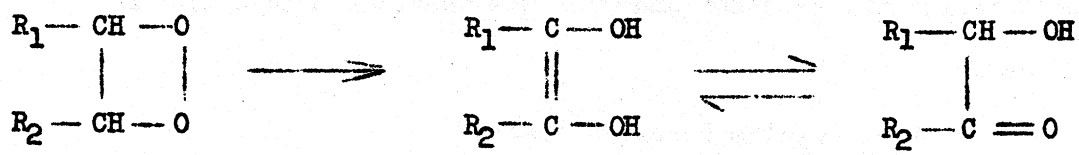
Any explanation of the process of autoxidation must begin, as in all related oxidation reactions, with an understanding of the nature of the first reaction of oxygen with the double bond system. Until this initial step is known with certainty the subsequent steps of the process must remain more or less speculative. It is for this reason that every theory which has been evolved with regard to the autoxidation of fats has been founded on some concept concerning this initial reaction and upon the chemical nature of the product thus formed.

a. Cyclic Peroxide Theory

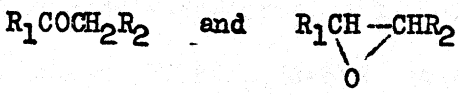
Although the autoxidation of fatty materials had been studied over one hundred years ago by De Saussure[14] and by others[15,16] shortly thereafter, the first observation of autoxidation of a carbon-to-carbon double bond has been attributed to Schönbein[17] who also investigated the reactions of a variety of oxidizing agents with unsaturated substances, such as almond oil and turpentine. The modern theories of autoxidation, however, are generally assumed to date from about 1900 with the work of Bach[18] and of Engler and co-workers[19-21] who investigated the role of organic peroxides in slow oxidation processes and introduced the term "activated oxygen". Prior to these, molecular oxygen was presumed to be broken down, at least to a small extent, into atomic oxygen in a manner analogous to the liberation of oxygen from hydrogen peroxide, and that this "active oxygen" was presumed to be responsible for the slow oxidation observed in various unsaturated organic substances. Bach and Engler, however,

believed that autoxidation by atmospheric oxygen was molecular and not atomic in nature, that is, a molecule of oxygen added at the double bond to form a peroxidized compound corresponding to the formula $R_1 - O - O - R_2$ and that this compound, like hydrogen peroxide, could in turn oxidize another oxidizable substance. The "activated oxygen" was not considered to be a free oxygen atom but was, rather, chemically bound in such a manner that it could readily be liberated to enter into autoxidative reactions.

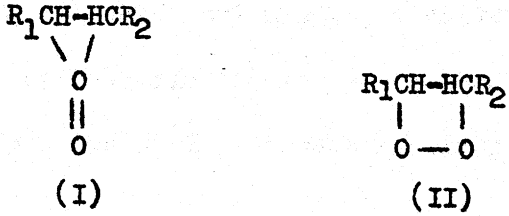
In an attempt to explain the action of driers in the formation of linseed oil films, Fahrion[22] and later Ellis[23] assumed that an autoxidation occurred in the fatty acid part to form a cyclic peroxide which underwent rearrangement to a dihydroxyethylenic or a hydroxyketo configuration as follows:



It was also presumed that the cyclic peroxide might give rise to more stable products corresponding to the formulas:



Staudinger[24] proposed a theory of autoxidation based on the assumption that the oxidation reaction originally proposed by Bach and by Engler was probably not the first but the second step in the autoxidation of ethylenic compounds. He assumed that a molecule of oxygen added at the ethylene bond to form a moloxide, probably corresponding to formula I, which subsequently underwent a rearrangement to form a cyclic peroxide, II:



(I) (II)

This theory was predicated on an investigation of the autoxidation of asym-diphenylethylene, $(C_6H_5)_2C{:}CH_2$. Diphenylethylene peroxide was isolated and found to be relatively stable, whereas the product of first addition of oxygen, which could not be isolated, was unstable and exploded when heated in a steel bomb to 40-50°C. Subsequently, it was learned that the isolable peroxide was a polymer and not a cyclic peroxide.

Although the majority of workers in the field accepted, at least until very recently, the theory of the formation of a heterocyclic peroxide of the formula

as the primary step in the autoxidation process, all the evidence for the existence of such peroxides was indirect, and no product containing this grouping had ever been isolated or identified from an oxidized fat or fatty acid. The existence of cyclic fatty peroxides was assumed on the basis of certain analytical data which were interpreted as substantiating the existence of such structures in autoxidized unsaturated acids. These analytical determinations included iodine, thiocyanogen, and diene values, total absorbed oxygen, peroxide value, molecular weight, saponification, hydroxyl, and carbonyl values. If the autoxidized acid or ester contained a single oxidation product only, and the methods gave quantitative and reliable results, the structure of the primary autoxidation product might have been deduced on the basis of these data, at least for the simpler unsaturated acids. As a general rule, however, all of these methods were applied to unfractionated, autoxidized acids or esters (and even to fats) in which oxidation had proceeded to the point where there existed in the reaction mixture a number of oxidation, degradation, and polymerization products. Consequently, the interpretation of the analytical data was extremely complicated. Furthermore, the methods were not entirely quantitative or specific in their application, especially in the presence of the considerable number of oxidation and degradation products which existed in the reacting system.

b. Ethylene Oxide Theory

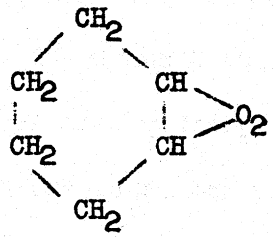
In 1909, Fokin[25] proposed a theory in which the first step in the autoxidation of an ethylenic bond was the formation of an ethylene oxide ring:

$$-CH=CH- + 1/2\ O_2 \longrightarrow -CH(O)CH-$$

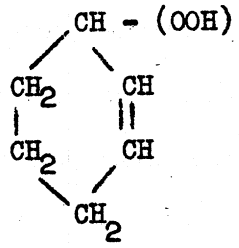
Fatty acids or esters containing the ethylene oxide (oxirane) ring have also been called epoxides, monoxides and oxido compounds. This structure is now known to be formed during epoxidation of unsaturated fatty materials with perbenzoic, performic, peracetic and other peracids, and many of these pure epoxy derivatives have been isolated and characterized.[26,27] Although epoxy compounds have been isolated from autoxidation mixtures,[23,28] it is doubtful that they are primary products. Experimental work in support of the ethylene oxide theory is the least extensive of any of the proposals, and it is no longer seriously considered by investigators in the field of autoxidation.

c. Hydroperoxide Theory

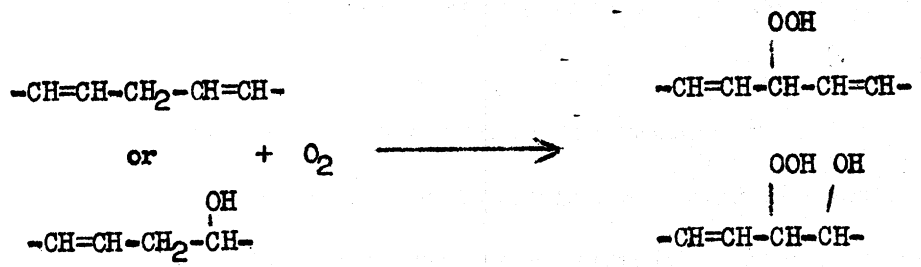
Much information regarding the mechanism of autoxidation of compounds derived from fats has been obtained by studying the oxidation of simple, monounsaturated, non-fatty compounds, such as cyclohexene, which can be prepared readily in a high degree of purity. In 1928, Stephens[29] reported the isolation of a peroxide of cyclohexene, $C_6H_{10}O_2$, which he obtained by treating cyclohexene with oxygen in daylight. He assumed, on the basis of the theories of oxidation accepted at that time, that the product was saturated:



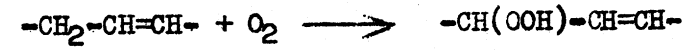
Further research, however, by Criegee and co-workers,[30,31] Hock[32-35] and notably by Farmer and Sundralingam,[36] established the fact that Stephens' product was a hydroperoxide and that a double bond was present:



It was also determined by Farmer's group that 1-methyl-1-cyclohexene and 1,2-dimethyl-1-cyclohexene behaved similarly when autoxidized. The actual isolation of purified hydroperoxides from these and many other autoxidized olefins was a tremendous step forward, and it cast much doubt on the validity of the older concepts of olefin oxidation. Rieche[37,38] postulated that unsaturated fats and oils probably behaved similarly. He suggested that the autoxidation of mono- or polyunsaturated substances may occur through the formation of oxygen-activated methylene groups according to the following scheme:



To Farmer and his co-workers,[39-43] however, is due the major credit for developing the hydroperoxide hypothesis of autoxidation, especially in its application to fatty acids, and for substantiating it with convincing experimental evidence. According to Farmer, the autoxidation of practically all unconjugated olefinic compounds proceeds by a chain reaction involving addition of a molecule of oxygen to the carbon atom adjacent to the double bond to form a hydroperoxide having an intact double bond:



As we shall see, this is probably not the primary step, but is the main chain-propagating reaction.

The hydroperoxide concepts are discussed in detail under the headings to follow, namely, autoxidation of monounsaturated fatty compounds, autoxidation of nonconjugated polyunsaturated fatty compounds, autoxidation of conjugated polyunsaturated fatty compounds and autoxidation of saturated fatty compounds.

i. Autoxidation of Monounsaturated Fatty Compounds

The autoxidation of pure monounsaturated fatty acids and esters although autocatalytic is slow at ordinary temperatures (below 60°C.). Ultraviolet radiation, metal catalysts and higher temperatures have been used to speed up the reactions. The rate of oxygen absorption of pure methyl oleate, methyl linoleate, and methyl linolenate has been shown to be about 1:10-12:16-25, at comparable temperatures.[44,45] Quantitative data of this type serve to confirm the hydroperoxide theory since if oxygen added to the double bonds exclusively, as had been proposed by so many earlier workers, the relationship just described should be closer to 1:2:3.

The early studies of reaction mechanism concentrated on the isolation of reaction products usually after extensive autoxidation had occurred. These investigations served to confirm the complexity of autoxidation but did not reveal the nature of the initial reactions.

In a logical extension of the earlier work on pure monoolefins of relatively low molecular weight, Farmer and Sutton[41] isolated nearly pure methyl octadecenoate hydroperoxides from methyl oleate autoxidation mixtures which had been oxidized in the presence of ultraviolet to low peroxide levels. Molecular distillation and chromatographic adsorption were used. The hydroperoxide was shown to contain about the theoretical peroxide oxygen content.

Catalytic hydrogenation yielded a mixture of monohydroxystearates and partial reduction with aluminum amalgam yielded a mixture of hydroxy-octadecenoates, thus demonstrating the presence of a double bond in the peroxide. Oxidation of methyl elaidate under similar conditions also yielded hydroperoxides.[46]

Later, Swift, Dollear and O'Connor[47] employed low temperature solvent crystallization to obtain a 90% peroxide concentrate from autoxidized methyl oleate. More recently, Fugger, Zilch, Cannon and Dutton[48] by counter-current distribution and also Privett, Lundberg and Nickell[49] by a modified extraction procedure have fractionated autoxidized methyl oleate between aqueous ethanol and hydrocarbon solvents and obtained 80-90% peroxide concentrates. Zilch and Dutton[50] also examined numerous model compounds known to be present in autoxidation mixtures. For large scale laboratory preparations, Coleman, Knight and Swern[51] employed the urea complex separation technique to precipitate unoxidized methyl oleate thereby concentrating the peroxides in the filtrate. Hydroperoxide contents of about 90% were consistently obtained if the extent of autoxidation did not exceed 15-20%.

According to Farmer, methyl oleate yields a mixture of mono- and dihydroperoxides (the former predominating) in which the hydroperoxide group is attached to the eighth or eleventh carbon atom. As Ross, Gebhart and Gerecht[52] and others[53,54] have shown, however, the hydroperoxide group is attached to the 8,9,10 or 11 position:

$$CH_3-(CH_2)_x-CH=CH-\underset{\displaystyle OOH}{\underset{|}{CH}}-(CH_2)_y-COOCH_3$$

$$CH_3-(CH_2)_x-\underset{\displaystyle OOH}{\underset{|}{CH}}-CH=CH-(CH_2)_y-COOCH_3$$

$$(x + y = 13)$$

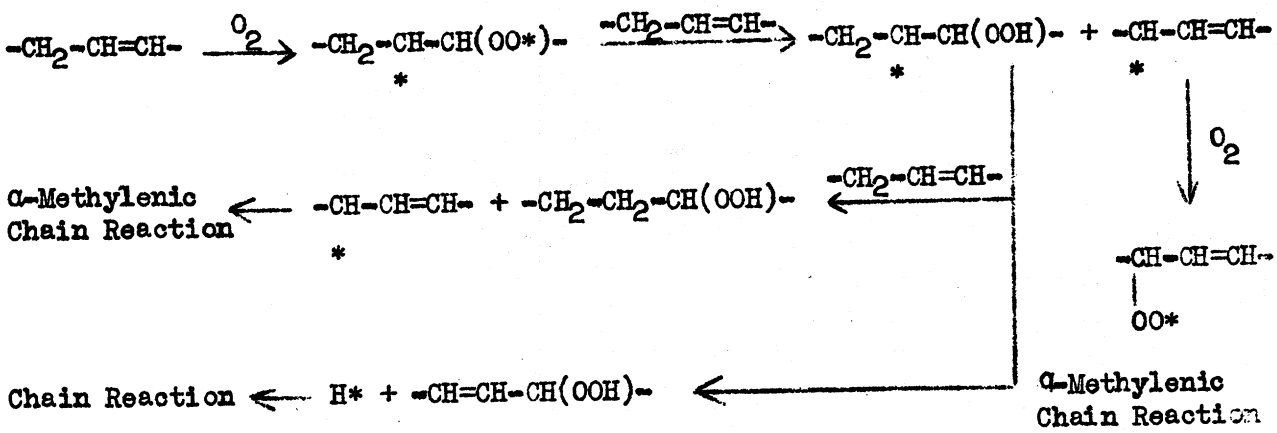
Based on the available evidence[54] it has been proposed that the hydroperoxides consist largely of methyl 9-hydroperoxido-trans-10-octadecenoate and methyl 10-hydroperoxido-trans-8-octadecenoate.

Although it has been assumed (tacitly perhaps) that the peroxides from autoxidized methyl oleate are exclusively hydroperoxides, it is doubtful that such is the case. Willits, Ricciuti, Knight, and Swern[55] have shown that polarographic analysis is a convenient and accurate way to determine hydroperoxides in the presence of other peroxide types. Polarographic examination of many peroxide concentrates from autoxidized methyl oleate has shown that, although hydroperoxides predominate, analytically significant amounts of non-hydroperoxides are also present. Polarographic studies of autoxidized methyl oleate and other materials have also been reported by Lewis and Quackenbush,[57] Nogami, Matsuda, and Nagasawa,[59] Paquot and Mercier,[60] Willits, Ricciuti, Ogg, Morris, and Riemenschneider,[61] and Saunders, Ricciuti and Swern.[62]

The structure of these nonhydroperoxides is still unknown. It is possible that they may be the cyclic peroxides which earlier workers proposed. The fact that reduced peroxide concentrates show an α-glycol content substantially equal to the original nonhydroperoxide content has been offered as evidence that cyclic peroxides are formed.[56] Until they are isolated, however, the evidence must be regarded as circumstantial particularly since α-ketols have been reported as components of the autoxidation mixture and these are also readily reduced to α-glycols.[63]

The facile formation of hydroperoxides from methyl oleate, and other olefins, had prompted Farmer and other investigators to propose that hydroperoxides are the initial products of autoxidation. Because of the energy required to rupture an α-methylenic C-H bond, and for other reasons,[6,11] Farmer,[39,64] Bolland and Gee,[65] and Gunstone and Hilditch[66] more or less

simultaneously concluded that the initial point of oxidative attack was at the double bond and <u>not</u> at the α-methylene group. It was agreed that double bond attack must occur to only a minor extent, probably in sufficient amount to "trigger" the α-methylenic chain reaction which predominates by far, as shown:



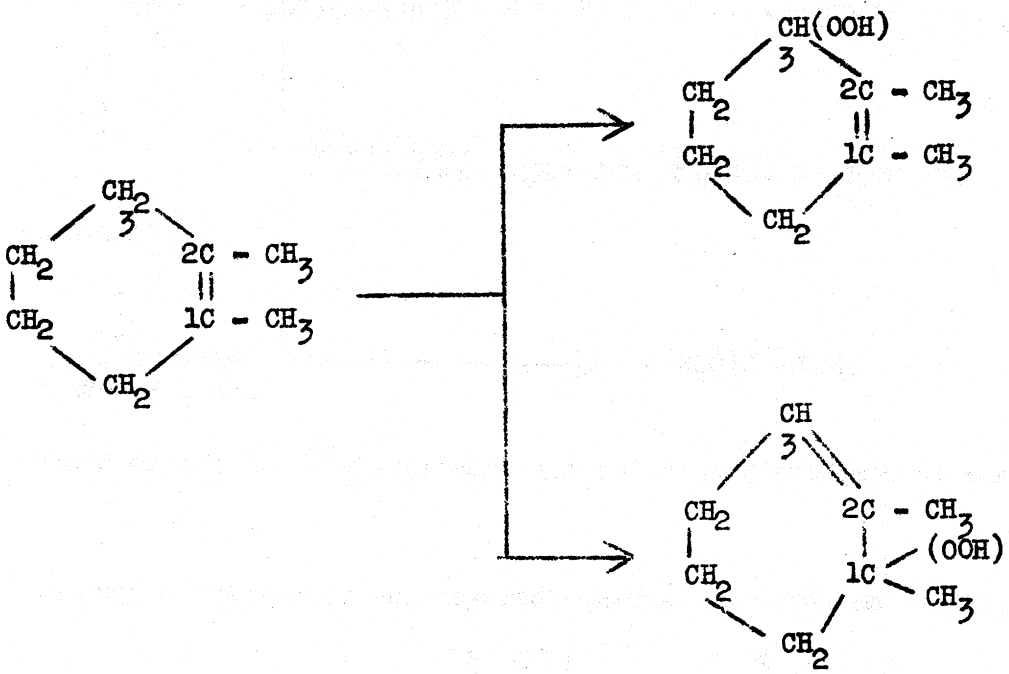
This reaction scheme is completely satisfactory kinetically[65] and thermochemically.[65]

In the free radical mechanism, resonance between the three-carbon systems

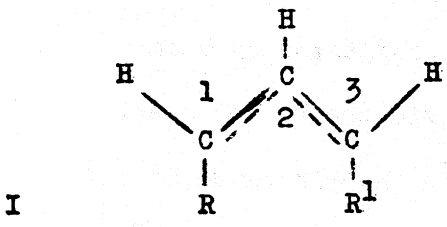
$$\overset{1\ 2\ 3}{\text{-CH-CH=CH-}} \quad \text{and} \quad \overset{1\ 2\ 3}{\text{-CH=CH-CH-}}$$

will occur[43] and when a molecule of oxygen and an atom of hydrogen are incorporated into the oxidizing molecule, there should be an approximately equal tendency for the hydroperoxide group to appear at positions 1 and 3, thus fixing some of the double bonds in the original position and the remainder at the adjacent pair of carbon atoms. This shift of the double bond, which had been demonstrated spectrophotometrically by Farmer, Koch, and Sutton[43] in the case of polyunsaturated compounds (discussed later), was suggested by them for monoolefins, although at that time no experimental evidence was offered. Evidence for this conclusion was subsequently obtained in the air oxidation of purified methyl oleate.[52-54] Additional chemical evidence that a double bond

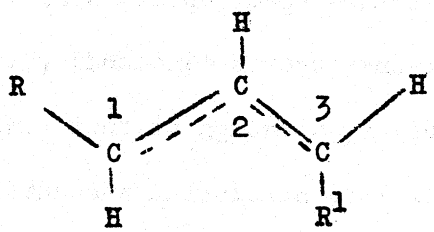
shift occurs in monolefin oxidations and, therefore, that a free-radical mechanism is probably applicable in these and comparable oxidations, was also supplied by Farmer and Sutton.[53] They studied the oxidation of 1,2-dimethyl-1-cyclohexene and concluded that both 1,2-dimethyl-1-cyclohexene-3-hydroperoxide and 1,2-dimethyl-2-cyclohexene-1-hydroperoxide must have formed:



Recently it was demonstrated by infrared observations that in the early stages of autoxidation of methyl oleate under ultraviolet light, peroxide formation is accompanied by the appearance of <u>trans</u> double bonds in an amount approximately 90% of the peroxide formed.[54] Among the possible mechanisms for this isomerization, the following was given. In the free radical formed, the atoms probably lie in a plane providing maximum resonance energy. The radical could then have two isomeric forms:



I



II

addition of oxygen to carbon 1 of either I or II or carbon 3 of I would yield a cis-hydroperoxide. Infrared observations suggest that most of the radicals assume the configuration II and add oxygen to carbon 3 yielding a mixture of trans-octadecenoates. This preferential attack may be favored by steric factors.

Under mild conditions and during the initial stages, the mechanisms of autoxidation favored at the present time have been described. These are probably supplanted, at least in part, by other mechanisms at later stages of the autoxidation, at higher temperatures and in the presence of catalysts. These conditions are conducive to accelerated peroxide decomposition, and the numerous radicals formed alter the course of the oxidation to one in which predominant attack may be at the double bond.

ii. Autoxidation of Nonconjugated Polyunsaturated Fatty Compounds

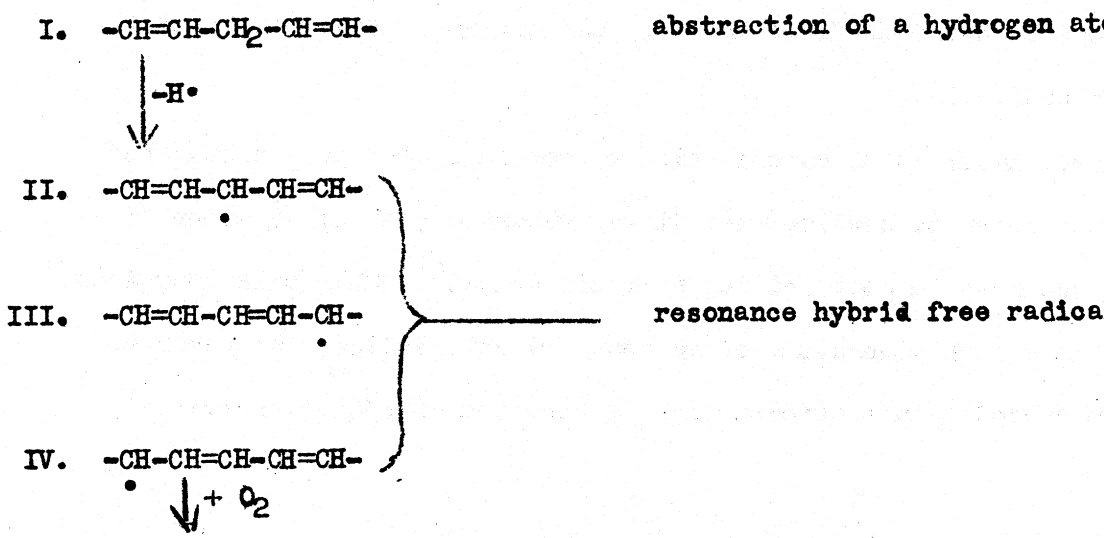
The rate of oxidation of methylene-interrupted polyunsaturated systems is much higher than that of monoethenoic systems because of the activation of a methylene group by two adjacent double bonds. This double activation results in oxidation rates twenty to forty times as great as in singly unsaturated compounds, making the polyethenoic acids the main source of oxidative rancidity problems. The oxidation of the polyunsaturated hydrocarbons, dihydromyrcene, dihydrofarnesene, and squalene was studied by Farmer and Sutton[67] who showed that during the early stages of oxidation substantially all the absorbed oxygen was in the form of hydroperoxides and that the original unsaturation of the compounds was unaffected.

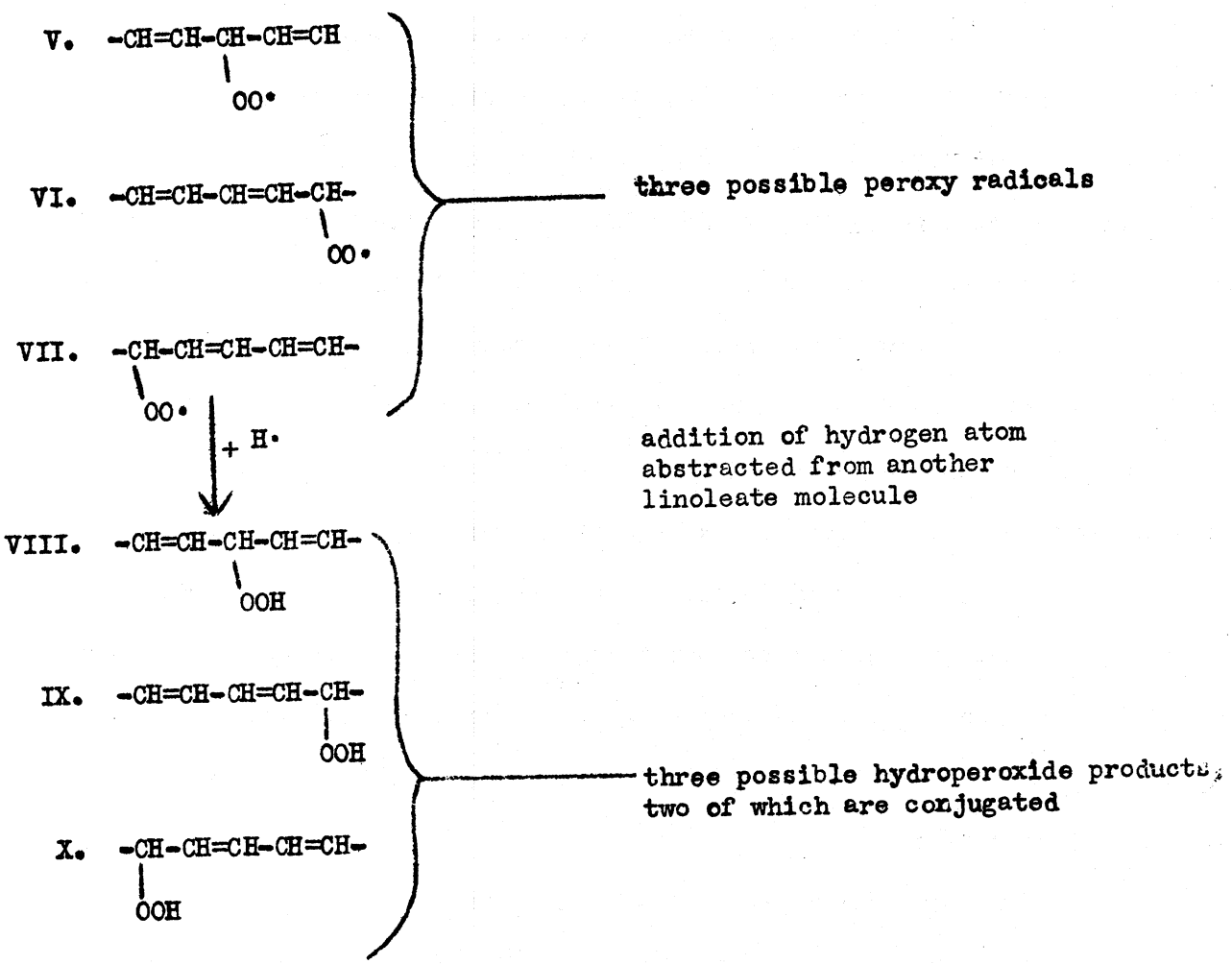
The current theories of autoxidation of nonconjugated polyunsaturated fatty compounds began to develop when it was observed that conjugation of double bonds occurred in autoxidizing fish oil acids.[41] When oils containing linoleate or more highly unsaturated systems are autoxidized, the diene conjugation as measured by ultraviolet light absorption at 2340 Å increases

parallel with oxygen uptake and peroxide formation in the early stages of oxidation. That this light absorption is not due to peroxide structure has been shown in an experiment in which thermal decomposition of peroxide did not diminish the absorption.[68]

The spectral changes occurring in autoxidizing fatty materials have been independently observed by several investigators and have been studied in considerable detail. The spectral changes accompanying oxidation are qualitatively similar for fatty acids containing two or more double bonds interrupted by methylene groups. Oxidized linoleate has a principal absorption at 2300-2360 Å, due to diene conjugation, and a secondary smooth absorption maximum at 2600-2800 Å, probably due to small amounts of unsaturated ketones. The principal bond is the same for linoleate, linolenate, and arachidonate, but the greater the degree of unsaturation, the lower the diene conjugation absorption per mole of absorbed oxygen. Conversely, the more unsaturated the fatty ester, the greater the light absorption caused by secondary reaction products.

In the oxidation of ethyl linoleate the monohydroperoxide which forms was shown by ultraviolet absorption measurements to contain approximately 70% of conjugated diene isomers.[68] This calculation was based on spectral data available at that time. The mechanism of reaction originally proposed by Bolland and Koch[68] is shown:





The value of 70% conjugation was taken as supporting evidence for the concept of random attack of oxygen on the free radical from linoleate, giving rise to three products, two-thirds of which were conjugated diene hydroperoxide. Bergström,[69] however, has separated the hydrogenated products of linoleate oxidation by chromatography. He isolated and identified 9- and 13-hydroxystearates but was unable to detect any of the 11-isomer which would have been produced if oxygen attacked the resonance hybrid at random. These results do not prove conclusively that the 11-hydroperoxide had not formed because under the conditions of hydrogenation, rearrangement of the nonconjugated hydroperoxide to conjugated isomers could have taken place.

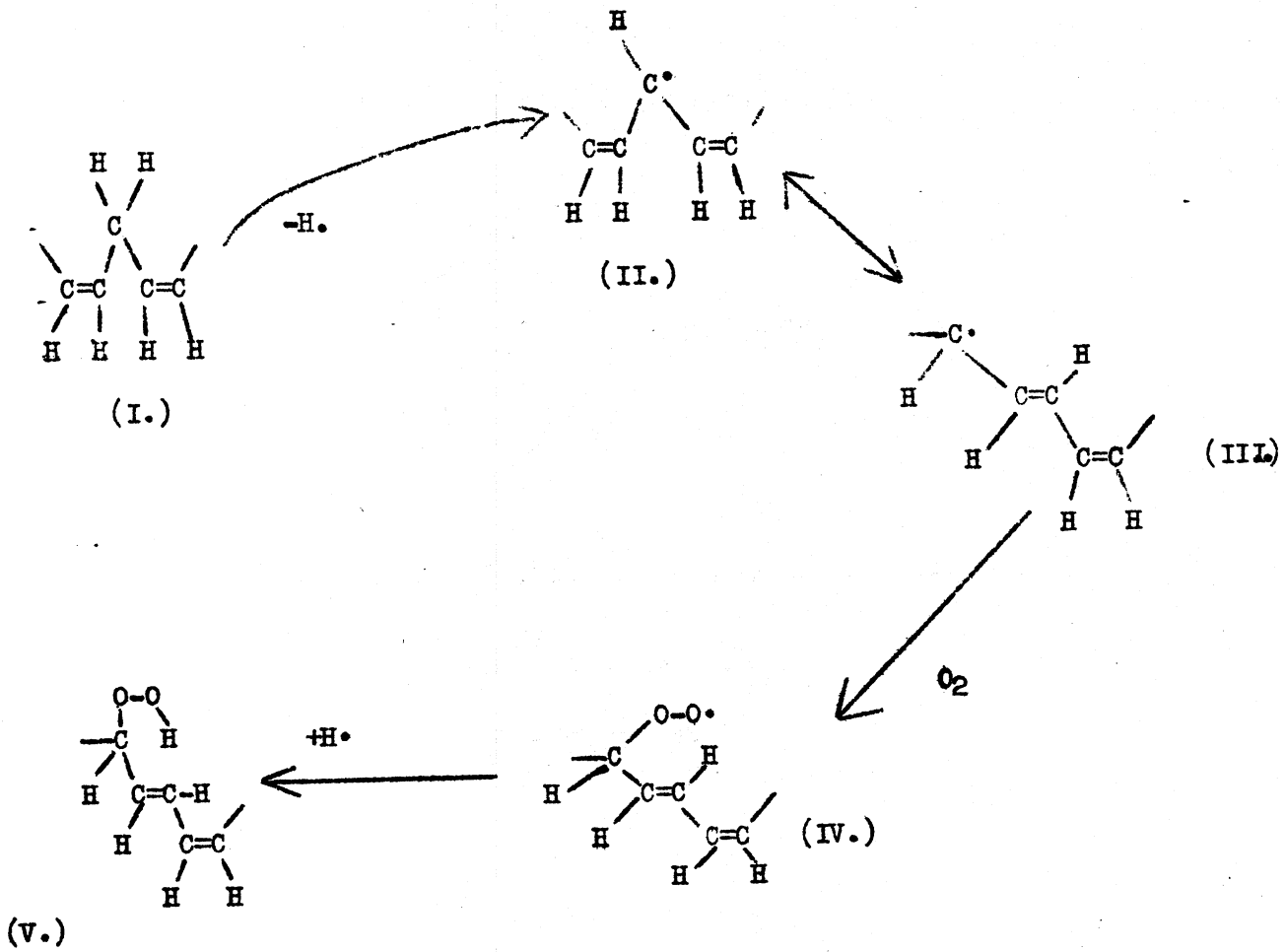
Until very recently only thermodynamic evidence had been offered in support of Bergström's evidence for the formation of conjugated hydroperoxide in excess of random amounts.[70,71] Khan, Lundberg and Holman[72] have obtained chromatographic evidence to support Bergström. Methyl linoleate was oxidized either by autoxidation in the dark at - 10°C, under visible or ultraviolet light, or in the presence of copper catalyst. The peroxides developed were separated by countercurrent extraction and then reduced by stannous chloride. These hydroxylinoleates were then separated by displacement chromatography. In the above-mentioned examples the products consisted almost entirely of conjugated compounds, but in the case where oxidation was stimulated by chlorophyll and irradiation a nonconjugated product was isolated. These observations suggest strongly that the conjugation observed was not induced by the reduction, and that autoxidation results largely in conjugated products. Sephton and Sutton have obtained similar results.[72a]

Much of the uncertainty regarding the true extent of conjugation produced arises from lack of proper standards of comparison. The estimates in the literature are based upon comparison with trans, trans-10,12-octadecadienoic acid which has a molar extinction coefficient of about 32,000. However, the effect of the peroxide group in the molecule, and the effect of cis, trans isomerism were not taken into consideration in making these comparisons.

Recent infrared spectral studies on the cis, trans isomers of linoleic acid[73] indicate that the conjugated linoleate hydroperoxide is not trans, trans and that estimates of the degree of conjugation present in the product must be revised upward. Recent studies of conjugated linoleate isomers obtained by alkali isomerization of linoleate indicate that these isomers have lower extinction coefficients at 2320 Å than the better known trans, trans isomers.[74,75] Privett and co-workers[76] and Dutton and co-workers[77] have

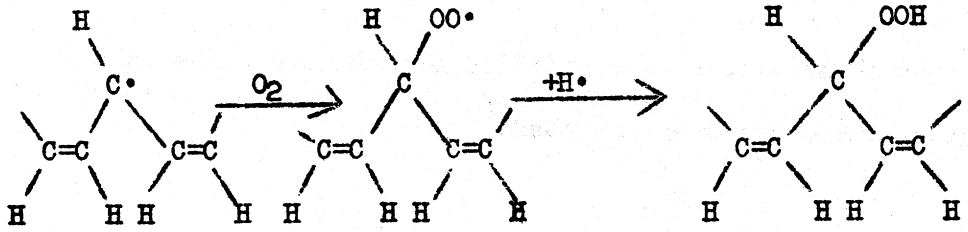
recently studied the infrared spectra of linoleate hydroperoxide preparations of high purity and have found that the product is at least 90% conjugated and that it consists largely of cis, trans isomers. The degree of conjugation calculated from ultraviolet absorption of the peroxide and known conjugated cis, trans linoleate also indicated at least 90% conjugation. These high degrees of conjugated cis, trans hydroperoxide were found only in preparations oxidized near 0°C. Oxidation at 24°C yielded peroxide concentrates in which appreciable amounts of conjugated trans, trans forms existed. The authors suggested that conjugated cis, trans isomers were initially formed and that the thermodynamically more stable conjugated trans, trans isomers arose from them through some catalysis possibly by peroxides.

In the light of these observations, a simplified mechanism for the main course of linoleate autoxidation has been proposed:[4]

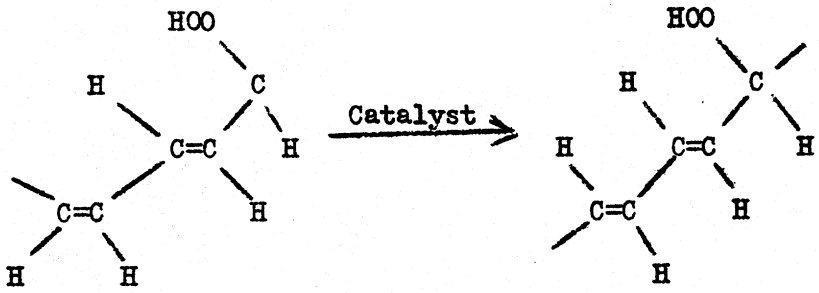


Linoleate, I, loses a hydrogen atom to some radical and becomes a free radical II. This free radical is a resonance hybrid, the two extreme forms of which are shown as III. Oxygen adds to the resonating radical, predominantly at the ends of the resonating system to yield two types of hydroperoxy radicals, IV. These radicals accept hydrogen atoms from other linoleate molecules to become isomeric conjugated cis, trans hydroperoxides, and in so doing perpetuate the cycle. Other reactions which occur to a limited extent under ideal conditions should be mentioned:

(1) Oxygen may add to the intermediate form of the free radical II to yield non-conjugated peroxides:



(2) The conjugated cis, trans linoleate peroxide may be isomerized to a trans, trans form:



(3) Polymers may be formed by addition of radicals II, III, IV with each other:

$$ROO\bullet + R\bullet \longrightarrow ROOR$$

$$R\bullet + R\bullet \longrightarrow R\text{-}R$$

The mechanism of formation of trans double bonds during autoxidation may be similar to that during alkali isomerization.[77a]

The cyclic or chain nature of the reaction is well established. The entire mechanism, however, involves three types of reactions: chain initiation, chain propagation, and chain-stopping reactions. The reaction chain can be initiated by the attack of any free radical upon linoleate. The most probable radicals to initiate chains are those formed by decomposition of a peroxide. It was formerly believed that peroxide was not required for chain starting, for Bolland[77] had shown that ethyl linoleate had a low but measurable rate of oxidation at 0% oxidation. Lundberg and Chipault,[78] however, demonstrated that highly purified linoleate had a long induction period, that is, it had no measurable oxidation rate for several hours after exposure to oxygen. The first chain must thus be initiated by some non-peroxidic free radical or by stray radiation. Parallel oxidation chains are initiated by radicals formed by decomposition of hydroperoxide. The higher the concentration of peroxide the more rapid is its rate of decomposition. Thus as the oxidation proceeds it generates its own catalyst. Hence the autocatalytic nature of the reaction. The reaction chains can be stopped by collision of two radicals, for example:

$$R\cdot + R\cdot \longrightarrow RR$$

$$R\cdot + ROO\cdot \longrightarrow ROOR$$

$$ROO\cdot + ROO\cdot \longrightarrow ROOR + O_2$$

If the radicals attack molecules of other substances present, products may be formed which do not decompose to form radicals and are thus incapable of propagating the reaction chain.

The reader is also referred to the work of Hilditch[79] whose conception of the reaction mechanism is not in agreement with that presented here, and to Gibson[80] who presents the mechanism of oxidation in a rather unique manner.

In the case of ethyl linolenate[43] there should be two initial radical forms (I and II) which could rearrange to III, IV, V and VI. The hydroperoxides derived from III through VI would then show diene conjugation. If both the active methylene groups were attacked consecutively by oxygen, which would happen frequently only in advanced stages of oxidation, then numerous diperoxide forms would become possible, some of which would show diene conjugation, some triene conjugation, and one the original state of unconjugation. The autoxidation of linolenates is thus even more complicated than that of linoleates. The autoxidation shows the characteristics of a chain reaction, hydroperoxides form, and double bond migration occurs but the fine details have not yet been developed.[8] At 0°C, 60% of the products are monomeric cis, trans-conjugated diene monohydroperoxides.

16 15 14 13 12 11 10 9

$-CH=CH-\overset{*}{C}H-CH=CH-CH_2-CH=CH-$ ⟶

I

$-\overset{*}{C}H-CH=CH-CH=CH-CH_2-CH=CH-$

III

$-CH=CH-CH=CH-\overset{*}{C}H-CH_2-CH=CH-$

IV

$-CH=CH-CH_2-\overset{*}{C}H-CH=CH-CH=CH-$

V

$-CH=CH-CH_2-CH=CH-CH=CH-CH=CH-$ ⟶

II

$-CH=CH-CH_2-CH=CH-CH=CH-\overset{*}{C}H-$

VI

Methyl docosahexaenote,[41,43] which is an extremely complex substance, also displays an increased absorption in the ultra-violet region, indicating that conjugated compounds are formed, but no quantitative data have been reported on this material.

In the case of squalene and rubber[43] no large increase in ultra-violet absorption which could be ascribed to the formation of conjugated units was observed. In these materials the reactive α-methylene groups are flanked on only one side by a double bond. This leads to a much lower reactivity of the olefin and requires two peroxidations to occur in any diene unit of the chain, -C=C-C-C-C=C-, before conjugation can appear. There is little doubt, however, that double-bond migration is occurring. It is unfortunate that the ultra-violet absorption technique is inapplicable to such systems.

iii. Autoxidation of Conjugated Polyunsaturated Fatty Compounds

The autoxidation of conjugated compounds has received much less study than that of the nonconjugated polyunsaturated fatty compounds, even though the former are important constituents of tung oil, oiticica oil and dehydrated castor oil which are widely used in protective coatings. The mechanism of oxidation of conjugated polyunsaturated fatty compounds is different from that of the non-conjugated, and the reaction products are not the same.

Studies with model conjugated hydrocarbons by Kern and co-workers[82-86] has shown that oxidation occurs by addition of oxygen to the diene systems to yield polymeric noncyclic peroxides mainly, although monomeric cyclic peroxides can also form in some instances. Both 1,2- and 1,4-addition occur depending upon the diene hydrocarbon. Hock and Siebert,[87] however, have reported the formation of both monomeric and polymeric cyclic peroxides by 1,4-addition, as well as simultaneous α-hydroperoxide formation.

Pioneering work in the autoxidation of conjugated polyunsaturated fatty compounds was done by Morrell and co-workers,[88,89] and by Miller and Claxton.[90] The former group, unfortunately, investigated an extremely complex system, namely, the adducts of methyl eleostearate with maleic anhydride. Both Morrell's group and Miller and Claxton carried the oxidation to advanced stages and

followed the oxidation by analytical means which, as we now know, is unreliable. They concluded that oxygen-containing polymers were formed and that ketol and enol groups were present in the products.

Brauer and Steadman[91] studied the course of oxidation of β-eleostearic acid by means of spectrophotometric measurements. They observed that the light absorption in the 2600-2800 Å region due to conjugated triene, decreased as the oxidation proceeded and that absorption due to conjugated diene increased. This has been found to be true also for pseudoeleostearic, α-eleostearic, and β-licanic acids.[92] Allen, Jackson, and Kummerow[93] compared the oxidation of 9, 12- and 10,12-methyl linoleate and found that in the early stages of oxidation of 9,12-linoleate, all the oxygen absorbed was found as peroxide, whereas in the oxidation of the conjugated isomer, no peroxide accumulated in the first stages of the process. The disappearance of conjugated double bonds was equivalent to oxygen absorbed, suggesting that carbon-to-oxygen polymerization occurred rather than carbon-to-carbon polymerization. The work of Jackson and Kummerow[94] on oxidation of the two linoleate isomers in the presence of metallic naphthenate driers indicates that the driers had less effect upon the oxidation of conjugated linoleate than upon nonconjugated linoleate. This would also suggest that peroxide decomposition is not a major factor in the mechanism of oxidation of conjugated substances.

The oxidation of conjugated unsaturated substances is accompanied by less breakdown than is the oxidation of the nonconjugated. The conjugated triene fatty acids and esters oxidize at a faster rate than do their nonconjugated isomers. This was shown by Myers, Kass, and Burr[95] who compared the oxidation of small amounts of trienoic acids and esters on filter paper. Comparison of the rates of oxidation of linoleic acid and 10,12-linoleic acid, however, showed no essential difference.[96] On the other hand, oxidation of the conjugated methyl

linoleate proceeded slower than oxidation of methyl linoleate in the experiments reported by Allen, Jackson and Kummerow.[93]

The most thorough report on eleostearate oxidation is that of Allen and Kummerow.[97] They found that the amount of triene conjugation lost and the amount of diene conjugation formed were both proportional to oxygen absorbed. The primary product of oxidation was isolated by low temperature crystallization and found to possess strong conjugated diene absorption in the ultraviolet. The effect of alkali upon this absorption was minimal. Hydrogenation of this oxidation product yielded mostly methyl dihydroxystearates. Oxidation of these with alkaline permanganate yielded only valeric and azelaic acids, indicating that the original oxidative attack had been confined within the triene system. The attack of oxygen upon the conjugated system was postulated to be 1,2-, 1,4-, or 1,6- yielding the following possible partial structures:

I. $R\text{-}CH{=}CH\text{-}CH{=}CH\text{-}\underset{\bullet}{CH}\text{-}\underset{\bullet}{CH}\text{-}R$

II. $R\text{-}\underset{\bullet}{CH}\text{-}CH{=}CH\text{-}\underset{\bullet}{CH}\text{-}CH{=}CH\text{-}R$

III. $R\text{-}\underset{\bullet}{CH}\text{-}CH{=}CH\text{-}CH{=}CH\text{-}\underset{\bullet}{CH}\text{-}R$

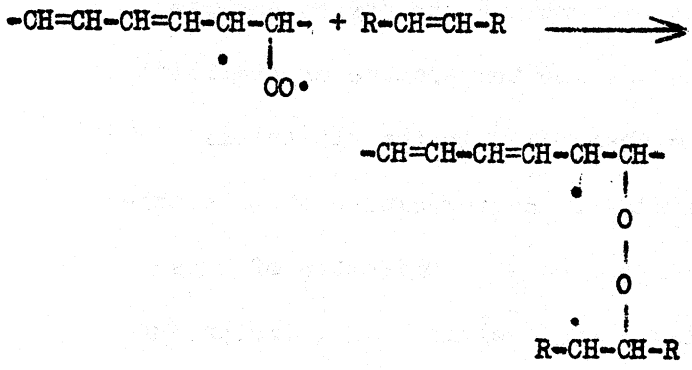
Two of these three possess residual diene conjugation. The isolated primary product of oxidation had a specific extinction coefficient of 62, compared with a value of 71 obtained by calculation. Upon hydrogenation at least three isomeric dihydroxystearates were obtained, only one of which contained an α,β-dihydroxy group. In accord with these chemical data and a kinetic study of the reaction, the following mechanism was proposed:

$$CH_3(CH_2)_4CH{=}CH\text{-}CH{=}CH\text{-}CH{=}CH\text{-}(CH_2)_7COOH + O_2 \longrightarrow$$

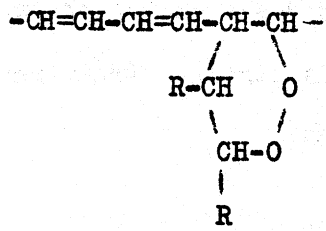
$$CH_3(CH_2)_4CH{=}CH\text{-}CH{=}CH\text{-}CH\text{-}\underset{|}{CH}\text{-}(CH_2)_7COOH$$

$$O\bullet$$

This diradical is stabilized by resonance along the unsaturated system, allowing addition at any of the carbons of the triene system. Reaction with another unsaturated molecule would yield a dimer still possessing free radical centers:



This dimeric diradical could stabilize itself internally to give a cyclic peroxide



However, the dimeric radical could again add oxygen and olefin thus building a polymeric chain in which the repeating unit is -CHR-CHR-OO-. Oxidation of pure material would favor the latter course whereas dimer formation would be favor in diluted media. The kinetic studies allow the rate of reaction of eleostearate oxidation to be summarized by the equation:

$$dO_2/dt = K(\text{product})^{1/2}\ (\text{ester})$$

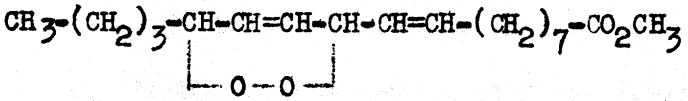
The rates of oxygen uptake for various conjugated unsaturated systems diminish markedly when the total oxygen uptake approaches 2 moles of oxygen per mole of ester or acid. This is true even for α- and β-parinaric acids which possess a conjugated tetraene system.[98] Apparently polymer formation increases the steric hindrance making further oxidative attack difficult.

Increasing viscosity and diminished diffusion of oxygen is apparently a lesser factor, for Chipault, Nickell and Lundberg[99] found that trieleostearin, pentaerythritol eleostearate and an eleostearic alkyd all became hard at a very early stage of oxidation, long before the maximum oxygen uptake had been achieved. On the other hand, the films set and hardened at later stages in the oxidation of similar linoleate and linolenate compounds.

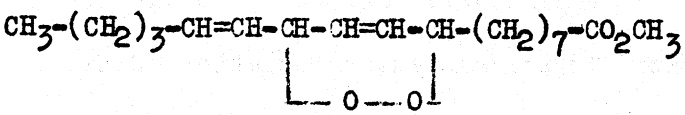
The reaction mechanism quoted above does not account for all the observations concerning conjugated polyene oxidation. The oxidation of the eleostearates has been shown to be autocatalytic both by Brauer and Steadman[91] in solutions, and by Myers, Kass and Burr[95] in thin films. According to the diradical reaction mechanism of Kummerow and his associates, propagation of the chain reaction is by polymerization only. The formation of small molecule products such as the dimers found by Brauer and Steadman is by early termination of the reaction chains. Chain reaction leading to monomeric or dimeric products could be only via a monoradical mechanism. Such a mechanism would involve the abstraction of a hydrogen from an eleostearate molecule.

The mechanism as postulated does not provide for autocatalysis. The chain polymerization reaction is not autocatalytic. Moreover, it is implied that polymerization is primarily through carbon to oxygen bonds, whereas it is known that eleostearate sets a hard film very early in the process of oxidation, before sufficient oxygen has been absorbed to account for the cross linking. Thus the polymerization is probably mostly through carbon-to-carbon linkages. It does not appear that the free radical reaction mechanism for conjugated polyene oxidation is obligatory. The simple 1,2- or 1,4- addition of oxygen may account for many of the facts, except that it does not account for autocatalysis. It would explain the partly conjugated primary product of oxidation isolated by Allen and Kummerow, and early polymerization could be stimulated by peroxide catalysis. The reaction mechanism is by no means settled.

More recently, O'Neill[7] studied the autoxidation of methyl eleostearate exposed to ultraviolet. When 1.2 atoms of oxygen had been absorbed per mole of substrate, it consisted of unchanged ester (38%), crystalline peroxide (7%), monomeric peroxidic, hydroxylic and ketonic products (35%), polymers (15%) and cleavage products (5%). Analysis of the crystalline peroxide suggested that it consisted of about 75% of an easily reducible 1,4-cyclic peroxide (I or II) and about 25%, of a more difficultly reducible and probably isomeric peroxide.



(I)



(II)

The remaining monomeric fraction consisted of other peroxidic material including about 15% of 1,2-cyclic peroxide, probably some 1,6-peroxide and hydroxylated and ketonic material not yet identified.

The polymeric material, the yield of which was unexpectedly low considering that a tung oil film will dry at an oxygen uptake of about one atom per fatty acid radical, was of higher molecular weight (number average about 1000) than the polymeric material obtained from experiments with the nonconjugated esters, and light scattering measurements suggested the presence of some high polymeric material. It contained peroxide groups which could not be readily hydrogenated and did not react with potassium iodide but which reacted with hydrogen iodide. The reaction with hydrogen iodide, however, yielded only small amounts of monomeric material, indicating that the chains were not linked by peroxide groups but were carbon-carbon bonded.

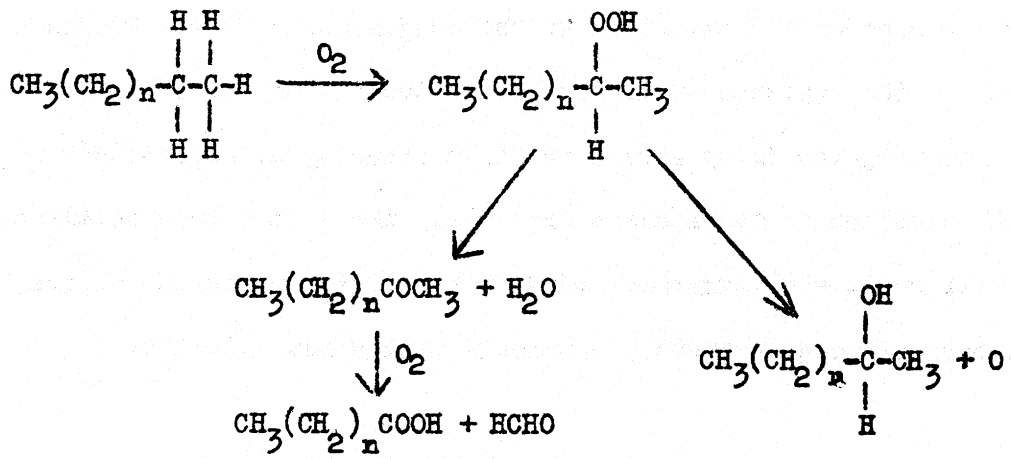
On oxidation of nonconjugated fatty esters, conjugated double bond systems are developed and the later stages of oxidation of these esters would be expected

to follow the same course as the oxidation of the conjugated esters. Evidence has been obtained for the presence of difficultly reducible peroxides in the autoxidation of nonconjugated fatty esters by first reducing hydroperoxides by hydrogenation with platinum at atmospheric pressure. The system then contains peroxides which will react with hydrogen iodide but not with potassium iodide, and which can be hydrogenated to hydroxy compounds with Raney nickel at 7 atmospheres.

iv. Autoxidation of Saturated Fatty Compounds

Under the mild conditions usually employed in autoxidation, saturated compounds have often been assumed to be inert. It is true that the main problems of autoxidation are associated with unsaturated fatty acids, but it is now known that saturated fatty acids undergo a slow autoxidation particularly at or above 100°C. Stirton, Turer and Riemenschneider[44] have shown that at 100°C the ratio of rate of oxygen absorption of methyl stearate: methyl oleate: methyl linoleate: methyl linolenate is 1: 11: 114: 179. A large amount of work has been published on the autoxidation of pure saturated hydrocarbons, because such studies are important for petroleum lubrication problems, and information obtained in this field is undoubtedly applicable to saturated fatty acids.[100-103a]

At 110°C saturated normal hydrocarbons autoxidize, the rate increasing with chain length. Thus, n-decane and n-hexadecane[101] absorb oxygen slowly during the early stages of autoxidation (the first 20 hours), and then an autocatalytic acceleration to a constant rate occurs. Autoxidative attack is probably random,[103a,104] and the primary product is a hydroperoxide.[100,102,-103,103a,104a] Decomposition of the hydroperoxide yields a ketone, an alcohol, or products of chain cleavage, as illustrated:



Attack can also be made at other carbon atoms, giving rise to other species of compounds. The aldehydes, ketones, and acids among the reaction products are subject to further oxidation and polymerization. As an example giving some conception of the rate of these oxidations, cetane absorbs approximately 0·002 mol oxygen per hour at 110°C. There is a slight increase in rate of oxidation with increased chain length. Branched and normal paraffins show similar autoxidation curves, having typical induction periods. The reaction is autocatalytic and is subject to acceleration by metallic ions and to inhibition by either metal-binding compounds or by antioxidants. The reaction is believed to be a chain reaction.

The autoxidation of saturated fatty acids or derivatives has received detailed study by Paquot and de Goursac.[105] Ethyl palmitate and ethyl caprate were blown with air at 120°C using 1% of nickel phthalocyanine as catalyst. About 40-50% of the original esters were recovered unchanged, 0.5% was lost as carbon dioxide, 5% was lost by entrainment, and the remainder was autoxidized. Sodium and potassium soaps have also been autoxidized by these investigators. The principal products were shorter chain acids and oxalic acid, thus suggesting that β-attack predominated. Minor amounts of lactones were also isolated indicating some δ- and γ-attack. Methyl ketones were also isolated in even smaller amounts.

It can probably be concluded, that during the early stages of the "drying" process the saturated components of an oil play little or no part in the reactions but that as oxygenated components accumulate, particularly peroxides, the rate of reaction of long-chain saturated compounds begins to be significant. In fact, the oxidation of the saturated components of an oil may play an important role in the ultimate deterioration of paint films and similar coating materials.

d. Kinetics of Autoxidation

Accurate comparisons of oxidation rates for various unsaturated fatty acids and esters cannot be made from the literature, for much depends upon the temperature and the manner of relating time with peroxide data. A number of investigators have shown, however, that the linolenates oxidize approximately twice as fast as the linoleates.[44,45] This may be correlated with the fact that linolenate has twice as many active methylene groups as has the linoleate. In oleate, where there are no methylene groups between unsaturated carbons, the oxidation is reported as ranging from one twelfth to one fiftieth that of linoleate. When the double bonds in fatty acids are separated by more than one methylene group, the reaction rate for each double bond approaches that of monounsaturated acids.

Allen[93] found that when the active methylene group is missing, as in conjugated $\Delta^{10:12}$ linoleic acid, the oxidation rate is one third that of the nonconjugated $\Delta^{9:12}$ linoleic acid. Furthermore, the $\Delta^{10:12}$ linoleic acid absorbed 1 mole of oxygen per mole of ester before any appreciable formation of peroxides took place. In compounds with more than two conjugated double bonds, however, conjugation increased the rate of oxidation. Myers, Kass, and Burr[95] found that conjugated triene esters oxidized more rapidly than nonconjugated triene esters.

The effect of the presence of small portions of linoleate upon the rate of oleate oxidation has been studied by Gunstone and Hilditch.[45] From these studies it is apparent that high purity of substrate is important in oxidation studies. Highly purified oleate esters are extremely resistant to autoxidation.[106]

In his kinetic studies on linoleate oxidation, Bolland[107] likewise studied the effect of ethyl linoleate concentration in ethyl oleate. He observed that the relative rate of oxidation bore a linear relationship to the molar concentration of linoleate. Thus the oxidation of the diluent, oleate, is not "catalyzed" by the presence of linoleate; the effect is one of dilution.

The more common unsaturated acids oxidize at maximum rates sometimes twice as great as that of their esters.[96] This effect is probably due to participation of the carboxyl groups in the decomposition of peroxides. This relationship is indicated by the work of Privett, Nickell, and Lundberg[49] in which addition of free linoleic acid to methyl linoleate peroxide accelerated its decomposition. Total oxygen uptake for free oleic acid has also been found to be less than for its esters.[108,109]

Although the preceding qualitative information on the effect of structure on rates of autoxidation is interesting and important, quantitative kinetic data are much more meaningful. One of the earliest kinetic studies was that of Henderson and Young[110] who derived the following rate law for the autoxidation of oleic acid:

$$\frac{-dO_2}{dt} = k_1 + k_2 \text{ (peroxide) } (O_2)^{1/2}$$

For oxygen pressures between 0.5 and 1.0 atmosphere the average value for k_1 was 2.2 and for k_2 226.

The detailed and complete studies of the kinetics of autoxidation described by Bolland, Bateman and co-workers,[1,2] however, are among the outstanding contributions to the mechanisms of autoxidation of unsaturated fatty materials.

These results domonstrate unequivocally that the low temperature liquid phase oxidation of olefins occurs by a chain mechanism. Thus, if inhibitors or initiators are added, spectacular decreases or increases in reaction rate, respectively, are observed. Also, if the reaction is accelerated photochemically, the quantum yield may exceed unity.

Comparison of the experimentally determined rate equations for the interaction of oxygen with olefins in the presence of benzoyl peroxide, ultra-violet radiation, or in the dark in the absence of added initiators shows an obvious parallelism in the way the rate of oxidation depends on $[O_2]$ and $[RH]$. Kinetic analysis shows that photo- and nonphoto-catalyzed oxidations proceed by the same basic mechanisms. The three rate equations reduce to the common form:[1,2]

$$\text{Rate} = R_1^{1/2} k [RH] \frac{[O_2]}{k' [RH] + [O_2]} \qquad (A)$$

(R_1 is the rate of formation of chain carriers)

The kinetic characteristics embodied in this generalized relation can be accounted for in terms of a single chain-reaction mechanism as follows:

INITIATION (Production of radicals R· or RO_2·) R_i

Benzoyl Peroxide
or $RO_2H + h\nu$ ⟶ R· or RO_2· $k_7(R_7)$
or $2RO_2H$ $k_1(R_1)$

PROPAGATION

$R\cdot + O_2 \longrightarrow RO_2\cdot$ $k_2(R_2)$

$RO_2\cdot + RH \longrightarrow RO_2H + R\cdot$ $k_3(R_3)$

TERMINATION

$$\left.\begin{array}{ll} R^{\bullet} + R^{\bullet} & \\ R^{\bullet} + RO_2^{\bullet} & \longrightarrow \text{Inactive Products} \\ RO_2^{\bullet} + RO_2^{\bullet} & \end{array}\right\} \begin{array}{l} k_4(R_4) \\ k_5(R_5) \\ k_6(R_6) \end{array}$$

The rate of oxidation is related to the velocity coefficient of the various elementary reactions (k_1-k_7) by

$$\frac{-d[O_2]}{dt} = R_i^{1/2} \frac{k_3}{\sqrt{k_6}} [RH] \frac{k_2 \sqrt{k_6}\,[O_2]}{k_3 \sqrt{k_4}\,[RH] + k_2 \sqrt{k_6}\,[O_2] + \sqrt{k_4 k_6 R_i}} \quad (B)$$

The correspondence between the experimental (A) and the theoretical (B) relations is complete since the term $\sqrt{k_4 k_6 R_i}$ becomes negligible at chain lengths as great as those encountered in these autoxidations.

The efficiencies with which the initiation process (R_1) and the termination reaction (R_6) occur are sensibly the same for a variety of nonconjugated unsaturated hydrocarbons. The resultant influence of the propagation and termination reactions involving the R-type chain carrier is negligible. The remaining chain-propagation step (R_3) is sufficiently sensitive to the nature of RH to introduce considerable variations in oxidation-chain length; R_3 must thus be regarded as the key reaction in controlling rates of autoxidation. Further details and discussion of these equations are given in Bolland's review.[2]

Chlorophyll also accelerates the autoxidation of oleic acid in the light and dark, but the mechanism of the reaction is not known.[107a]

Recently Khan, Brown, and Deatherage,[108] Max and Deatherage,[111] and Khan[112] studied and compared the rates of autoxidation of methyl oleate, methyl 9,10-dideuterooleate, and 8,8,11,11-tetradeutero-cis-octadecene. These investigators showed that deuterium compounds oxidize at a slower rate than do

the corresponding hydrogen compounds. They concluded that initial autoxidative attack is at the double bond, and the main sustaining reaction is attack at the α-position.

e. Secondary Products of Autoxidation

Numerous investigators have studied the secondary products of autoxidation of fatty materials. In the early studies of reaction mechanism the isolation of reaction products, usually after extensive autoxidation had occurred, was a major objective of many workers. It was hoped that the mechanism could be worked out by a careful separation and quantitative estimation of the products. The large number of products formed and the dependence of their yield on reaction conditions made this approach relatively fruitless, except to reaffirm the complexity of autoxidation. Unfortunately, in many of the early studies impure starting materials were chosen, thus complicating further an already complex situation.

One of the first systematic investigations was that of Skellon[113] who oxidized pure oleic acid at 100-120°C for long periods. He isolated two 9,10-dihydroxystearic acids, a monohydroxystearic acid, and numerous compounds arising from chain cleavage. Many years later, Feuell and Skellon[113a] reinvestigated the problem and showed that, in addition to the earlier reported products, oxiranes, unsaturated carbonyls and dimers formed. Ellis[23,28] oxidized oleic and elaidic acids until 1-3 moles of oxygen were absorbed at 55-80° with a cobaltous elaidate catalyst. Epoxide formation accounted for at least 20% of the products when catalyst was used. This was taken as evidence that oxygen attacked the double bond, but as we shall see later, this conclusion is not justified. Cleavage products included nonanoic, octanoic, suberic, azelaic, and oxalic acids, as well as carbon dioxide and water. Peroxides were minor products. Deatherage and Mattill[109] largely confirmed these results and showed

that epoxy derivatives were among the chief products of autoxidation. Swern, Knight, Scanlan and Ault[114] used molecular distillation to fractionate the products of an extensive cobalt salt-catalyzed autoxidation of methyl oleate and showed that oxygen-linked polymers were formed. Using acetic acid as a solvent and a cobalt salt-catalyst, Knight, Jordan, Koos and Swern[115] showed that oleic acid and methyl oleate gave 64-68% yields of mixed cleavage products (mono- and dibasic acids) and 12-17% of high-melting 9,10-dihydroxystearic acid. Thus about 80% of the starting material was accounted for but the cleavage products consisted of C_6-C_{12} mono- and dibasic acids, with no particular ones predominating.

Recently it was shown that in the uncatalyzed autoxidation of methyl oleate substantially all of it undergoes single attack by oxygen or peroxides before any significant quantity of multiple attack in the chain occurs.[116] A typical composition, after the peak in peroxide content has been passed, is about 30-35% peroxide, 25-30% hydroxy compounds, 20-25% oxirane compounds, 15-20% α,β-unsaturated carbonyl compounds and some residual methyl oleate, cleavage products, polymers, and multiply attacked methyl oleate. Hydrogenation of such mixtures with Raney nickel and palladium as catalysts yields monohydroxystearic acids in high yield.[117]

α,β-Unsaturated carbonyl compounds have been isolated by Ellis[118,119] from autoxidized oleic and elaidic acids. These products would appear to be obtained directly from the corresponding α-methylenic hydroperoxides simply by the loss of a molecule of water. 12-Ketoelaidic and 12-ketooleic acids, isomers of the α,β-unsaturated carbonyls obtained in autoxidation, have been prepared synthetically by controlled oxidation of ricinelaidic and ricinoleic acids.[119] 12-Ketoelaidic acid is relatively stable toward autoxidation whereas 12-ketooleic acid absorbs oxygen even at 0°C. This relative autoxidizability

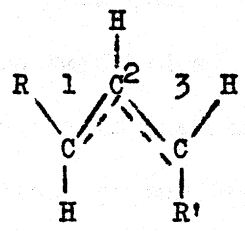
is of particular significance because it has been shown that most, if not all, of the hydroperoxides formed from methyl oleate have the trans configuration.[54] The α,β-unsaturated carbonyl compounds derivable from these hydroperoxides should also be trans since dehydration does not involve the double bond. Although the α,β-unsaturated carbonyls formed during autoxidation are not identical with 12-ketoelaidic acid they are closely related, and it might be assumed that they too would be resistant to further autoxidative attack in the absence of catalysts. The fact that single attack on the methyl oleate chain predominates supports this conclusion; otherwise a significant amount of multiple attack would occur before all the methyl oleate had been autoxidized.

It is known that oxirane compounds are also resistant to further autoxidative attack at moderate temperatures, and it would appear that the unsaturated hydroxy compounds must be too. Although this latter point has not been resolved experimentally, it is probable that the hydroxy compounds, as well as some of the oxiranes, are converted to esters, thereby enhancing their stability.

King[63] has recently published a detailed analytical study of the autoxidation of elaidic acid at 47°C and 78°C with and without a cobalt salt catalyst. The formation of ketol derivatives has been confirmed, and formic acid has been identified among the volatile products of autoxidation. Methods were also reported for estimating ketol and other carbonyl compounds.

Skellon and Thruston[119a] studied the catalytic oxidation of elaidic acid, methyl elaidate and n-propyl elaidate. About 33% peroxides formed with methyl elaidate at 98°C but only 10-15% with elaidic acid. (This parallels the experience with methyl oleate and oleic acid.) Decomposition of the hydroperoxides occurred along three lines: a) monomeric oily complexes containing carbonyl groups, b) other secondary products such as oxiranes and keto esters and c) cleavage products.

It was shown by Ellis[28] that both oleic and elaidic acids on autoxidation give trans-9,10-epoxystearic acid in about 20% yield. The fact that both a cis and a trans compound yield the same, rather than different, products by the identical oxidation method is indeed surprising and remained unexplained for many years. With the development of infrared spectrophotometric methods for determining trans compounds in autoxidized and other materials, it was shown that the majority of radicals in the autoxidation of oleic (and elaidic) acid take the configuration shown below,



and add oxygen at carbon atom 3.[54,115] The resulting hydroperoxides, 9-hydroperoxido-10-trans-octadecenoic acid and 10-hydroperoxido-8-trans-octadecenoic acid (I and II), on homolytic cleavage yield the radicals HO• and RO• (III and IV). Both III and IV, by shift of one π-electron of the double bond to couple with the odd electron on oxygen, would form the oxirane ring in the 9,10-position. Reacquisition of a hydrogen atom then yields trans-9,10-epoxystearic acid.

$CH_3-(CH_2)_6-CH=CH-\underset{\underset{OOH}{|}}{CH}-(CH_2)_7-CO_2H$ (I)

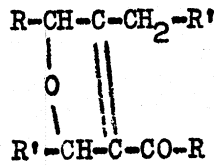
$CH_3-(CH_2)_7-\underset{\underset{OOH}{|}}{CH}-CH=CH-(CH_2)_6-CO_2H$ (II)

$CH_3-(CH_2)_6-CH=CH-\underset{\underset{O\bullet}{|}}{CH}-(CH_2)_7-CO_2H$ (III)

$CH_3-(CH_2)_7-\underset{\underset{O\bullet}{|}}{CH}-CH=CH-(CH_2)_6-CO_2H$ (IV)

This mechanism also accounts for the formation of high-melting 9,10-dihydroxystearic acid from both oleic and elaidic acids.

In the uncatalyzed autoxidation of methyl oleate, polymer formation does not occur until advanced stages.[116] When metal catalysts are present, however, polymers form even in the early stages. The structure of the polymers formed from autoxidizing methyl oleate is not known. Evidence has been published suggesting that they are largely oxygen-linked[7,114] as distinguished from methyl linoleate polymers in which carbon-carbon linkages are known to be present certainly in the dimers.[7,120] Ellis[118] has proposed that the dimers from autoxidized oleic acid are substituted dihydrofurans (V) which are formed from the α,β-unsaturated carbonyls:



(V)

Kern[120a] has pointed out that fatty chains are linked by C-O-C, C-O-O-C, and C-C bridges in autoxidatively produced polymers. No true chain propagation occurs because of a low polymerizing tendency in these systems and also because of the inhibiting action of oxygen.

Swift, Dollear, Brown, and O'Connor[121] have shown that one of the decomposition reactions of methyl oleate hydroperoxides is cleavage to α,β-unsaturated carbonyls, one of which is 2-undecenal. Swift and Dollear[122] have reported that oleic acid can be intermolecularly oxidized at 90° by methyl oleate hydroperoxides to form cis-9,10-epoxystearic acid, m.p. 59°, and low melting (but not high-melting) 9,10-dihydroxystearic acid, m.p. 95°, in small quantity.

Recently Ahlers and McTaggart[123] have devised infrared spectroscopic methods for the quantitative determination of secondary products of autoxidation, such as hydroxy and carbonyl compounds. The method requires only about 20 milligrams

of sample (which can be recovered after examination), and the accuracy of each determination is similar to that of the corresponding conventional chemical method.

Most of the modern published work on secondary products of autoxidation has been devoted to monounsaturated fatty materials. Swift, O'Connor, Brown and Dollear,[124] however, have identified one saturated and two unsaturated aldehydes as oxidation products from decomposed hydroperoxides of cottonseed oil. Linoleic acid is the principal fatty acid constituent as well as the most readily oxidized component of this oil. Johnson, Chang and Kummerow[124a] isolated methyl ethyl ketone, acetaldehyde, α-pentenal, crotonaldehyde and an unknown five-carbon carbonyl compound in the volatile decomposition products of the oxidative polymers of ethyl linolenate. Similar cleavage products have been isolated from autoxidized soybean,[124b,124c] corn and avocado[124d] oils and methyl linolenate.[124b]

f. Miscellaneous

Peroxide Catalysis - In the autoxidation of unsaturated substances, the induction period is that initial period of time during which no appreciable oxidation takes place, and during which peroxide does not accumulate rapidly. When peroxide (or partially oxidized material) is added to a fat it has a pro-oxidant action because, in effect, the stage of oxidation is advanced beyond the induction period. Peroxides, being unstable substances, have appreciable thermal decomposition rates, and thus provide free radicals to initiate new chains. Benzoyl peroxide is well known for initiation of polymerization reactions and has been used as a source of chain-starting radicals in studies of autoxidation, as has linoleate hydroperoxides,[65,107] peracids,[124e] free radicals,[124f] hydroperoxides and peroxides.[124g]

Metal Catalysis - In the drying oil industry, pro-oxidants called "driers" are often used to promote rapid oxidation. These substances are salts of heavy metals with organic acids. The effect of driers upon drying time of linseed oil has been extensively studied by Lund[125] and the action of copper upon the oxidation of linoleic acid has been studied by Smith and Stotz.[126] Skellon[127] studied the action of various metal salts upon oleate oxidation and found that lead, aluminum and barium are good catalysts in the primary stage of oxidation, but that zinc is a good catalyst for formation of ketone from peroxide. Linoleates were more effective catalysts than oleates, elaidates or ricinoleates.

Metallic catalysts have their action primarily through the decomposition of peroxides to form additional free radicals. No work in the field of fats has treated this phenomenon, but it is generally accepted that peroxide decomposition via metallic salts to yield free radicals promotes polymerization[128] and oxidation.[129] It is clear, however, from the study by Jackson and Kummerow[94] that in the presence of drier, the peroxide value of oxidizing linoleic acid is held to a lower level.

Many organic peroxides are able to take electrons from and yield electrons to metallic cations, depending upon oxidation reduction potential:

$$Cu^{+} + ROOH \longrightarrow Cu^{++} + R\text{-}O:^{-} + \cdot OH$$

$$Cu^{++} + ROO:^{-} \longrightarrow R\text{-}OO\cdot + Cu^{+}$$

The metallic catalysts function through maintaining a steady concentration of active hydroxyl radicals.

High polyvalency is not indispensable for good catalytic activity although valence changes are characteristic of the best ones.[129a] According to Skellon,[129b] catalysts appear to function through a simple cycle of valency changes.

1) A loose complex forms between metal catalyst, in a lower state of valency, and oxygen, the metal being raised to a higher valency condition. 2) This condition of valency is more stable but still highly reactive towards oxygen. In this state the catalyst reacts further with oxygen and oxidizable substance, donating oxygen continuously to the latter without permanently reverting to its lower valence state, that is, the catalyst-oxygen complex acts as an oxygen donor. 3) Ultimate interaction between catalyst in its higher valency condition and a quantum of oxidized material causes reversion of the metal to its lower valence state accompanied by changes in the character of the oxidized material.

Film formation with driers present is marked by a shortened induction period, an increase in rate of oxidation, film formation at a lower oxygen content than in absence of driers, and a lower total oxygen content during aging.[129a]

Andersson[130] presents a slightly different view of the metal catalyzed oxidation of methyl linoleate. In his work he found that after the induction period the rate increased to a constant value ($\underline{r}$) and could be related to drier concentration ($\underline{c}$) by the following relation:

$$r^2 + \alpha r = \beta c,$$

in which α and β are constants dependent upon linoleate concentration. The induction period increased with drier concentration, suggesting that it is related to the oxidation of the cations to a higher valence. Ninety per cent of the absorbed oxygen was found as peroxide. His conception of the mechanism of catalyzed and uncatalyzed autoxidation is the same except that the chain carrier is an addition product of the catalyst rather than the peroxide radical ROO•.

A survey of drier action and methods of manufacture were recently published.[130a,130b]

Surface Catalysis - The influence of the nature of the vessel upon induction periods and rates of oxidation has been known for some time. The more common surface catalysis is due to contact with atoms of the transition metallic elements in the vessel, and is thus ordinary metal catalysis. However, George[131] has made a systematic study of surface catalysis by means of the addition to the test sample of inert powders which contained subanalytical amounts of transition element impurities. In common with metal catalyzed and benzoyl peroxide catalyzed oxidation, the surface catalyzed oxidation of tetralin yielded hydroperoxide as the primary product. The surface catalysis is responsible for initiation and termination of reaction chains.

Antioxidants - Antioxidants are substances which are able to prevent or delay the oxidation of the oil, when present in small quantities. They are present in most natural fats and oils, and contribute to the natural stability of raw oils which is often removed by purification. The literature on antioxidant theory and practice has been reviewed by Lundberg up to 1947.[132]

The mechanism of inhibition catalysis was recognized by Alyea and Bäckström[133] to be the breaking of reaction chains and involved the oxidation of the inhibitor. Bolland and ten Have[134] studied the kinetics of ethyl linoleate oxidation in the presence of hydroquinone (Hq) and came to the conclusion that the inhibitor terminates chains by interaction with peroxide radicals:

$$R\text{-}OO\cdot + Hq \longrightarrow \text{Stable Product}$$

From kinetic evidence they also concluded that hydroquinone underwent chemical change. The strong yellow color of the oxidized mixture suggested that the product of hydroquinone oxidation was benzoquinone. Golumbic[135] found that

tocopherol was rapidly oxidized during the induction period of fat oxidation, and when tocopherol had disappeared, the induction period came to an end. Lundberg, Dockstader, and Halvorson[136] studied the kinetics of oxidation of hydroquinone, catechol, nordihydroguaiaretic acid, and gallic acid in oxidizing lard. They found that in each case, antioxidant concentration diminished during initial stages of oxidation and that peroxide did not reach high values until most of the antioxidant had disappeared.

Taylor[137] and Michaelis[138] have discussed the mechanism of antioxidant action. Michaelis explained antioxidant phenomena on the basis of compulsory univalent oxidation-reduction. Aside from synthetic sulphur compounds, two types of natural antioxidants exist, one which can be reversibly oxidized to quinone, and tocopherol which cannot. They do have the common property of being reversibly oxidized to a semiquinone radical. Semiquinones are well known, and the semiquinone of tocopherol has been demonstrated to exist by Michaelis. Tocopherol in alcohol, ether and pentane was cooled to a glassy noncrystalline mass. When this rigid solution was irradiated with ultraviolet light, an intense red color was produced, which disappeared when the glassy solution melted, allowing dismutation of the free radicals. From these observations the most plausible mechanism of antioxidant activity is the following:

$$ROO\cdot + AH_2 \rightarrow ROOH + AH\cdot \qquad \text{primary attack}$$

$$2AH\cdot \rightarrow AH_2 + A \qquad \text{dismutation}$$

or

$$ROO\cdot + AH\cdot \rightarrow ROOH + A \qquad \text{secondary attack}$$

Synergists - Synergists are substances which reinforce the effect of antioxidants. The synergists may or may not possess antioxidant activities of their own. Synergists are usually dibasic or polybasic organic or inorganic acids. The synergists thus far receiving the most study are ascorbic acid, phosphoric

acid, and citric acid. Golumbic[139] stated that the function of a synergist was the continual regeneration of the antioxidant at the expense of the synergist which acted as a source of hydrogen.

In a current paper Privett and Quackenbush[140] point out that three factors are incompatible with that mechanism: (1) some potent synergist combinations do not form oxidation-reduction systems; (2) in the tocopherol-ascorbic acid synergism, no evidence has been presented to define the rate of ascorbic acid destruction; and (3) phosphoric acid has been shown to react with quinone in the absence of hydrogen donors to give a false positive test for tocopherol in the iron-bipyridyl reaction. Privett and Quackenbush found that citric and ascorbic acids suppress the initial accumulation of peroxides which takes place at "pro-oxidant" levels of nordihydroguaiuretic acid and tocopherol in autoxidizing lard. It was also found that ascorbic acid and tocopherol exerted sparing actions on each other in oxidizing lard; the tocopherol was not spared at the expense of ascorbic acid.

The work of Privett and Quackenbush indicates that synergists suppress the "pro-oxidant" action of phenolic antioxidants. This "pro-oxidant" action is a catalysis of peroxide decomposition stimulated by the antioxidant, particularly when it is in high concentration. In an autoxidizing fat containing both antioxidant and synergist, the antioxidant has two actions. The antioxidant terminates oxidation chains by reacting with peroxide radicals:

$$ROO\cdot + AH_2 \longrightarrow AH\cdot + ROOH$$

The inhibitor also catalyzes the decomposition of the peroxides, the extent of which is dependent upon antioxidant concentration.

$$ROOH \xrightarrow{AH_2} R\cdot + \cdot OOH$$

The function of the synergist is probably to suppress the antioxidant's catalysis of peroxide decomposition. By suppressing the catalysis, additional chain formation is prevented, and thus the antioxidant molecules are spared from their function in stopping such chains.

The reader who wishes more details on the mechanism of action of antioxidants and their practical evaluation should consult the recent review by Riemenschneider.[140a]

3. Quantitative Determination of the Products of Autoxidation

a. Peroxides

Quantitative determination of peroxides is the most commonly used analytical method for following the course of autoxidation reactions. Many methods have been described for determining peroxides; these have been discussed critically by Barnard and Hargrave[141] and several of the more important ones have been examined in a statistical manner by Ricciuti, Coleman and Willits.[142]

i. Iodimetry

Iodimetric determination of peroxides is the most popular. The methods are all based on the assumption that potassium iodide (or other salt) and hydriodic acid when brought in contact with fatty peroxides liberate iodine quantitatively in some simple stoichiometric manner, namely, 2 atoms of liberated iodine are equivalent to 1 atom of active oxygen.

The peroxide methods of Lea[143] and of Wheeler[144] are widely used, but recent studies show that improvement can be made by exclusion of oxygen from the reagents and reaction flask and also by excluding light.[145,145a] Because of its widespread use, the Wheeler method, with modifications, is described here.

Modified Wheeler Method.[144,145a,146] - Twenty milliliters of 3 to 2 acetic acid-chloroform are introduced into a glass-stoppered 250-ml. iodine flask. A weighed sample is transferred to the flask which is flushed with nitrogen.

Two milliliters of freshly prepared 50% solution of potassium iodide in water are added, and the flask is again flushed quickly with nitrogen. After 15 minutes, 50 ml. of water are added, and the liberated iodine is immediately titrated with 0.1N sodium thiosulfate. The entire procedure is so arranged that the contents of the reaction flask are not in contact with any appreciable amount of dissolved or atmospheric oxygen at any time until the water is added.

Under these conditions, the liberated iodine rises to a maximum concentration in less than 15 minutes, and thereafter remains constant. Peroxide values obtained with reaction times up to 2 hours are no different from those obtained in 15 minutes.

$$\% \text{ Peroxide Oxygen} = \frac{\text{ml. of thiosulfate} \times \text{normality} \times 0.008 \times 100}{\text{wt. of sample}}$$

ii. Polarography

Polarography has recently been used in the analysis of fat oxidation products. Lewis, Quackenbush, and De Vries[57,58] found a linear relationship between wave height and peroxide value in the early stages of the oxidation of fats.

A more detailed examination of the polarographic behavior of autoxidation products was carried out by Willits, Ricciuti, Knight and Swern.[55] These workers showed that peroxides, hydroperoxides, aldehydes, ketones conjugated with a double bond and α-diketones could be measured polarographically. In particular, they demonstrated that hydroperoxides could be determined quantitatively in the presence of other peroxide types.[142]

iii. Miscellaneous Peroxide Analytical Methods

Ferric Thiocyanate Method. - This method lends itself to colorimetry, but is subject to considerable error. Chapman and co-workers[147] and Erdmann and Seelich[147a] describe such a method, but the peroxide as determined by this

means is far higher than that found by iodometric methods. Lea[148] found that by exclusion of oxygen from the reaction medium the color formation is diminished to about one-fourth that obtained in the presence of oxygen, and that the values were lower than theoretical. The thiocyanate method has its use in comparative studies where a quick colorimetric method is desired, but it requires rigid exclusion of air for reproducibility. The values obtained in the presence of air are proportional to, but higher than iodometric values. It is more sensitive than the iodometric method and can be used in lower ranges of peroxide value.

Dichlorophenol-Indophenol Method. - This method, introduced by Hartmann and Glavind[149] likewise yields high values in the presence of air.[150] However, the values are reproducible and are useful only in comparative studies. Perhaps the most significant use of this reagent has been to detect the presence of peroxides histochemically.[151]

Stannous Chloride Method. - Barnard and Hargrave[141] have proposed a modification of the stannous chloride method which they believe to be satisfactory. The method is titrimetric and requires about 1 milliequivalent of peroxide for determination. A weighed sample containing 0.75 to 1.0 meq. of peroxide is dissolved in acetic acid (10 ml.) in a 250-ml. Erlenmeyer flask, which is then evacuated to 20 mm. of mercury and filled with nitrogen. Fifteen milliliters of 0.1N stannous chloride solution are added from a pipet, and the flask is immediately re-evacuated and filled with nitrogen, the latter procedure being repeated twice. After standing for 1 hour at room temperature, a boiling solution consisting of 5 ml. of stock ferric solution, 1 gram of ammonium chloride, and 45 ml. water is added. The mixture is kept at 75°C. for 30 seconds and then rapidly cooled, and 10 ml. of phosphoric acid solution are added. The ferrous ion is titrated with 0.05N potassium dichromate solution

and 6 drops of indicator solution (0.25% solution of diphenylamine sulfonic acid in water). The end point is a sharp transition from green to violet. Blank determinations are carried out in a similar manner.

$$\% \text{ Peroxide Oxygen} = \frac{(\text{blank} - \text{titer}) \times \text{dichromate normality} \times 0.008 \times 100}{\text{wt. of sample}}$$

b. Other Oxygen-containing Species

The determination of autoxidation products other than peroxides is not simple. Early investigators in the field failed to realize that conventional fat-analytical methods were not necessarily applicable to autoxidation mixtures. For this reason much of the early work on autoxidation, particularly the composition of the oxidation mixture, is open to serious question.

A detailed re-examination of analytical methods as applied to autoxidation systems was published by Knight and Swern,[152] and the modified procedures were applied to autoxidizing methyl oleate.[152a] It was shown that in the absence of peroxide and oxirane groups, the analytical procedures were reliable. When peroxides were present, as is usually the case, high and variable values for carbonyl oxygen were obtained, and the iodine and saponification numbers were generally unreliable. Determination of hydroxyl oxygen was interfered with by large proportions of oxirane compounds but apparently not by peroxides. Determination of acid number and peroxide and oxirane oxygen was reliable in the presence of all the other functional groups investigated. Techniques were reported for the accurate determination of functional groups when peroxide and oxirane groups were present. Skellon and Feuell[152b] have recently described methods for estimating ketonic groups in complex oxidation products.

Ahlers and McTaggart[124] have devised infrared spectroscopic methods of analysis which require only 20 milligrams of sample and the accuracy of each

determination compares favorably with that of the corresponding chemical method. This procedure offers much promise in the rapid, accurate analysis of small quantities of autoxidation products.

4. Miscellaneous Investigations

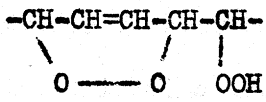
Acetylenic Compounds. - Although acetylenic fatty acids do occur in natural oils and these substances are subject to autoxidation, very little information has been gained regarding the mechanism of this oxidation. Khan, Deatherage, and Brown[108] have made a study of the autoxidation of stearolic acid and its methyl ester in comparison with oxidation of oleates. In contrast to oleates, stearolic acid and methyl stearolate had no induction period. The oxidation began at its maximum rate. The acetylenic compounds absorbed oxygen at a faster rate (4X) than did the oleates. Oxidation of stearolate was accompanied by considerable polymerization and the residues contained a considerable amount of carbonyl oxygen. No diketostearic acid was found, however. Acid and ester groups were present but only small amounts of peroxide and hydroxyl groups were present. The volatile products of oxidation of stearolate consisted of water, carbon dioxide, and other organic products. In the case of methyl stearolate, water evolved equalled more than 0.8 mol per mole substrate and carbon dioxide more than 0.1 mol per mole substrate. The nonaqueous volatile products of stearolate oxidation had a rancid odour but gave a negative Kreis test, whereas the corresponding product from oleate gave a positive test. These striking differences between the kinetics and products of oxidation strongly indicate a difference between the mechanism of oxidation of oleate and stearolate. Khan, Deatherage and Brown[108] suggest that α-methylene attack of acetylenic compounds is the predominant type of oxidation rather than addition to the double bond.

A study of the oxidation of matricaria ester (n-decadiene-2,8-diyne-4,6-oic acid methyl ester) by Holman and Sørensen[153] indicates that in this conjugated system the oxygen addition follows a biphasic curve. Likewise, the increase in light absorption at 3550 Å in alkaline solution increases rapidly initially, whereas the increase in absorption at 6000 Å corresponds to the second phase of oxygen addition. The oxidation was accompanied by rapid polymerization and intense deepening of color. The mechanism of oxidation of conjugated unsaturated systems involving triple bonds, such as occur in matricaria ester, and isano oil, is probably much different from that of isolated triple bonds. It is likely that the mechanism of oxidation of these mixed double and triple bond conjugated systems is similar to that of conjugated polyenes, for rapid polymerization occurs during their oxidation. One would expect oxygen to add to the two types of resonating systems in similar manners.

Physical State of Substrates. - The degree of dispersion of unsaturated substrates has a marked effect upon their oxidation. The spreading of oil films on porous material can, and often does, lead to spontaneous combustion of the oil. Honn, Bezman, and Daubert[154] have studied the oxidation of drying oils adsorbed on the surface of finely divided silica gels. There is a critical oil/solid ratio which yields a maximum rate of oxygen uptake. The results were interpreted as indicating the existence of a closely packed monomolecular layer of oil on the adsorbent at the critical ratio, and that this arrangement is most favorable for promoting oxidation. At oil concentrations below the critical oil/solid ratio, the oil molecules are separated by distances dependent upon the ratio, and thus the rate of oxidation depends upon the average distance between oil molecules. Above the critical ratio, the oil molecules form multimolecular films, and the rate of oxidation is decreased, because diffusion of oxygen becomes a limiting factor. This interpretation

was verified by the observation that at the critical ratio, the calculated area occupied by that amount of oil as a monolayer nearly equalled the area available on the silica gel surface.

In aqueous colloidal solution, sodium linoleate oxidation appears to be slightly different from oxidation of linoleate esters in bulk. Bergström, Blomstrand, and Laurell[155] found that in this system the rate of oxidation was dependent upon copper ions, that maximum oxygen uptake is 2 moles of oxygen per mole linoleate, and that the spectral changes were similar to those observed in oxidizing linoleate esters. Isolation of the reaction products as a low viscosity oil indicated that polymerization had been inhibited, and the sharp termination of the reaction at 2 moles of oxygen per mole linoleate suggests that the product of oxidation may be rather reproducible. The authors proposed that monomeric diperoxides of the structure



may be formed by the oxidation of the primary peroxide. This method should prove useful in preparation of the product of linoleate oxidation which has 2 moles of oxygen per molecule.

Emulsified oils are subject to oxidation, but because of the instability and nonreproducibility of emulsions, little work has been done on fat oxidation in this type of system, with the exception of enzymatic studies. However, emulsions are of biological and medical interest. Oil in the dispersed phase is subject to the same type of oxidation as oil in bulk, but the presence of water-soluble catalysts has an influence upon the oxidation. Metallic salts and haemoproteins are of particular importance as catalysts in oxidation of biological systems. Much of the catalysis in animal tissue fats which has been considered enzymatic is due to oxidation catalyzed by haemoglobin, myoglobin and catalase.[156]

Most work on fat oxidation has used liquid systems. Oxidation of solid fats is much inhibited by the presence of considerable amounts of saturated fatty acids which act as diluents. On the other hand, oxidation is sharply limited by the solid state of matter in which penetration and diffusion of oxidation is much reduced.

Restriction of unsaturated fatty acids and esters within the crystal structure of urea complexes is effective in inhibiting oxidation of these readily oxidizable substances. The inhibition of oxidation in this case could be either by protection against oxygen penetration, or by prohibition of chain reaction as a consequence of the rigid lattice to which the substrate is restricted.[157]

Oxidation in the gaseous phase is extremely rapid, but little is known of its mechanism. Contact of hot vapors of fatty acids or esters with air can lead to explosive oxidation.

Irradiation. - The oxidation of unsaturated acids and esters is stimulated by various types of irradiation. The absorbed radiant energy activates substrate molecules to the energy level necessary for chemical reaction to take place. Infra-red and visible radiation are somewhat effective, but ultraviolet light, because of its higher energy content, is far more effective. X-ray has recently been found to be effective in inducing fat oxidation.

Ultraviolet light has been used successfully by Farmer and Sutton[41] to promote the oxidation of methyl oleate to the hydroperoxide. Sutton[46] used the same method for oxidation of methyl elaidate to the hydroperoxide. Swift, Dollear, and O'Connor[47] were able to oxidize methyl oleate rapidly by ultraviolet light irradiation and to prepare the hydroperoxide in 90% purity.

Bateman and Gee[158] have made a thorough kinetic study of the photo-oxidation process, using cyclohexene, 1-methylcyclohexene, 2,6-dimethyl-2,6-octadiene, and ethyl linoleate as substrates. They concluded that

photo-oxidation proceeds by a chain mechanism in which the generation of free radicals by light absorption is the chain initiation mechanism. The predominant initiation process is the photolysis of the hydroperoxides. When light intensity is fixed and wavelength chosen such that light absorption is weak, the photooxidation is autocatalytic, because the products formed are more strongly light-absorbing than the substrate, and their photolysis leads to additional chain formation.

Photo-oxidation is somewhat modified by the presence of chlorophyll.[72,159] Khan, Lundberg, Tolberg, and Wheeler[159] have studied the chlorophyll-photo-oxidation of methyl oleate and methyl linoleate. The visible light energy absorbed by the chlorophyll is in some manner transferred to the substrate, thereby activating the substrate to an energy level sufficient for oxidative attack to take place. The products of linoleate oxidation have a lower light absorption in the ultraviolet region characteristic of conjugated dienes, whereas the products isolated from thermal and plain photo-oxidation are very similar in light absorption. Infra-red studies of the product suggest the presence of isolated trans material in the product, whereas this is not found in products of plain photo-oxidation. Chlorophyll photo-oxidized linoleate yielded a peroxide concentrate of high peroxide value, which after reduction to the alcohol and chromatographic separation yielded a significant fraction which was nonconjugated. These results suggest that the 11-hydroperoxido-octadecadienoate can exist and that by some means becomes a significant product by the action of chlorophyll in spite of the greater thermodynamic stability of the conjugated isomers.

Mead[160] has found in his studies on x-irradiation of linoleate that the reaction is also a chain reaction. He has measured the quantum yield and found that with increasing concentration, the ionic yield increases.

With increasing cysteine concentration, the ionic yield decreases, indicating protection of linoleate against oxidation by sulfydryl compounds. Presumably the mechanism of x-ray stimulated oxidation of linoleate is the same as in autoxidation. The possibility also exists that radiation-initiated chain oxidation of unsaturated fat accompanies radiation injury of animals.

Enzymes. - Lipoxidase is a plant enzyme specific for the oxidation of the essential fatty acids and their esters. The enzyme has been studied carefully, and for detailed information the reader is referred to recent reviews.[156,161,162]

Lipoxidase attacks essential fatty acids, yielding hydroperoxide as a primary product. The enzymatic oxidation of linoleate is accompanied by the complete conjugation of the double bonds and the formation of optically active hydroperoxides.[162a] The double bonds are cis, trans and trans, trans.[162a,163a] The enzyme is active in the range from the freezing point of the solution to somewhat above room-temperature, although the enzyme is inactivated during its action at the higher temperatures.

The most plausible reaction mechanism for linoleate oxidation by lipoxidase involves probable contact between the enzyme and each molecule of the substrate. Tappel, Boyer and Lundberg[163] also found that the enzyme was capable of oxidizing antioxidants in the presence of linoleate without the concomittant oxidation of linoleate itself.

The oxidation of polyphenolic antioxidants by lipoxidase plus linoleate suggests that linoleate may play a role analogous to a coenzyme or prosthetic group in the oxidation of polyphenols and other substances. The reported lipoxidase oxidation of amino acids and polyphenols may be by such a mechanism. If so, the enzyme may be as important in the oxidation of these secondary substrates as in the direct oxidation of polyunsaturated fatty acids.

Strain[163b] has pointed out that the direct catalytic formation of peroxides in the presence of oxidase occurs only with those compounds containing $-CH=CH-(CH_2)_7-CO-$ with a cis configuration.

Biological Effects of Autoxidized Fats. - The adverse effect of the ingestion of rancid fat is well known. The subject has been reviewed by Burr and Barnes[164] and by Quackenbush.[165] They concluded from the evidence at that time that one of the chief deleterious effects of dietary rancid fat is the destruction of vitamins and perhaps other dietary essentials. On the other hand, many symptoms observed are not explained in terms of vitamins, and at least for the present it must be assumed that autoxidized fat has a direct growth-depressing effect (see below).

Rancid fat in the diet causes the development of a biotin deficiency because of the oxidation of biotin which is synthesized in the intestine.[166] This is in agreement with observations that biotin is inactivated by rancid fats in vitro.[167] Ascorbic acid may be partially destroyed as a consequence of its synergistic antioxidant action. Pyridoxine and pantothenic acid have been found to relieve acrodynic rats fed rancid fat.[168] Tocopherol and carotene are easily oxidized; Vitamin D is subject to oxidation under conditions which prevail in dietary preparations.[169] Deficiencies in Vitamin K,[170] Vitamin A,[171] and riboflavin,[172] as well as the effect of cortisone,[173] are intensified by autoxidized fat. Fresh fat, however, exerts a protective effect on the toxicity of heated and aerated cottonseed oil.[174]

Destruction of Essential Fatty Acids. - It is of course obvious that if rancidity is caused largely by the oxidation of polyunsaturated fatty acids, these essential fatty acids are destroyed in the course of the process. Unfortunately, this aspect of rancid fat toxicity has had little attention until recently. In the study of the ability of rancid fat and oxidized esters to

relieve the symptoms of acrodynia - a deficiency disease caused by removal of pyridoxine and essential fatty acids from the diet - oxidized methyl linoleate relieved the symptoms but was not as effective in doing so as fresh ester. Tocopherol did not improve the response to the rancid preparations. Highly oxidized preparations were ineffective.[168]

It has been suggested that one role of tocopherol *in vivo* may be to provide an antioxidant status for the protection of essential fatty acids (polyunsaturated fatty acids). To test this hypothesis Witten and Holman[175] attempted to simulate pro-oxidant status by feeding benzoyl peroxide with the essential fatty ester supplement, and to simulate antioxidant status by feeding added tocopherol with the supplements. The conversions of linoleate and linolenate to more highly unsaturated fatty acids by fat deficient rats were used as an index of essential fatty acid utilization. It had been previously found that in rats, linoleate induces synthesis of arachidonate and linolenate induces synthesis of hexaenoate, with other cross conversions also taking place.[176] When benzoyl peroxide plus linoleate was fed as a supplement to fat-deficient rats, growth response was greatest, least when benzoyl peroxide was fed alone. Fat synthesis was found to be stimulated by benzoyl peroxide plus unsaturated ester. Benzoyl peroxide plus linoleate led to the formation of hexaenoic acid, but other conversions appeared to be unaffected by either tocopherol or benzoyl peroxide. Thus, contrary to expectation, the pro-oxidant, which is toxic when fed alone, proved to be beneficial according to criteria of growth and fat synthesis.

From the above results it appeared that oxidized essential fatty acids are involved in some of the conversions. To explore this question further, Holman[4] administered to fat deficient rats the following supplements: ethyl linoleate, partially oxidized ethyl linoleate, ethyl linoleate with dietary

benzoyl peroxide, ethyl linoleate hydroperoxide, thermally decomposed ethyl linoleate hydroperoxide, and conjugated ethyl linoleate. Fresh linoleate, oxidized linoleate and linoleate plus benzoyl peroxide all relieved the dermal symptoms of fat deficiency, reduced the water consumption of the rats, and stimulated arachidonate synthesis. Linoleate peroxide, decomposed peroxide, and conjugated linoleate were not effective as judged by these criteria. Thus the concentrated products of linoleate oxidation and conjugated linoleate could not be utilized as essential fatty acid. However, fresh linoleate, slightly oxidized linoleate, and linoleate plus dietary benzoyl peroxide were curative. The beneficial effect of feeding the catalyst for autoxidation, benzoyl peroxide, plus linoleate together in supplement suggested that a more oxidizable medium may be better utilized by the animal. The influence of pro-oxidant and antioxidant conditions upon essential fatty acid metabolism is still not clear, and it is obvious that much experimental evidence and verification will be needed before the matter is understood.

Biological Effects of Autoxidatively-Produced Polymers. - There is increasing evidence that the abnormal nutritional properties of highly autoxidized fats are related in part to the polymers which develop during autoxidation. Kaunitz, Slanetz, Johnson, Knight, Saunders and Swern[177,178] have isolated the polymers from highly autoxidized cottonseed oil and highly autoxidized lard and have fed them to rats. Diets containing 20% of autoxidatively-produced polymeric residue led to diarrhea and rapid death whereas with only 10% most of the animals were gradually able to tolerate it. At the 4 or 7% level, it was well tolerated but growth was reduced. There were no distinctive histological lesions and withdrawal of the polymer permitted immediate realimentation without evidence of subsequent injuries.

The polymeric residue from autoxidized cottonseed oil exerted a greater growth-depressant effect than that from lard. Addition of fresh fat to the polymeric residue decreased the growth-depressant effect. On diets marginal in protein, autoxidatively-produced polymers intensified the deficiency effect at polymer levels which had little or no effect in normal diets.[178]

Effect of Oxidized Fat on Enzyme Systems. - Bernheim, Wilbur, and Kenaston[178a] have recently demonstrated an inhibition of enzyme action by fatty acid oxidation products. Washed tissue suspensions or mitochondria, when incubated with ascorbic acid, lose enzymatic activity. The decrease in succinoxidase, cytochrome oxidase, and choline oxidase activities has been found to parallel the amount of oxidized unsaturated fatty acid as measured by the thiobarbituric acid test. The enzyme inactivation can be prevented by quercetin, which inhibits oxidation. Oxidized methyl linolenate also inactivates the enzymes. These observations are of interest particularly in the case of cytochrome oxidase, which is known to be associated with lipid containing some highly unsaturated acids. It may well be that the inhibition is due to the oxidative destruction of essential fatty acids which are a part of the active enzyme system.

Qualitative Detection of Oxidation Products. The Kreis Test. - This test has been used widely for the assay of oxidative status of oils, but it offers the disadvantages of being highly empirical. The test as ordinarily used involves two phase systems or other means of separation of the active components. Modification of the test making it suitable for colorimetry has improved the method.[179] Phloroglucinol has been shown to yield color with epihydrinaldehyde, malonic dialdehyde, or acrolein treated with H_2O_2,[180] but the presence of these compounds has not been demonstrated in oxidized fat, nor do these substances appear in the currently accepted mechanism of oxidation. Their presence, however,

is not unreasonable as secondary oxidation products. Thus the Kreis test, although a proven qualitative test for oxidation, has been of little theoretical value in studies of fat oxidation. It appears that the Kreis test detects substances formed from the decomposition of peroxides, and represents a measure of terminal oxidation products. It may yet prove to be of value in studies of secondary oxidations and peroxide decomposition.

Thiobarbituric acid Test. - This test has been developed in the past few years, and appears to be related to the Kreis test. It was originally used by Kohn and Liversedge[181] who observed that animal tissues, upon aerobic incubation, were able to give a color reaction with thiobarbituric acid. Bernheim and co-workers[182,183] have traced this reaction to products of oxidation of unsaturated fatty acids, principally linolenic acid. Patton and Kurtz[184] have studied the reaction involved in the test and applied the test to detection of oxidation in milk fat. They found that malonic dialdehyde gave a strong test and that the thiobarbituric acid test is much more sensitive than the Kreis test. The latter did not begin to yield measurable colors until after decided oxidized flavors appeared, whereas the thiobarbituric acid test gave measurable responses during the development of perceptible rancidity. Methyl oleate hydroperoxide also gives the color reaction.[184a] The thiobarbituric acid test appears, therefore, to hold more promise than does the Kreis test. It would be of value in detection of oxidation in its early stages.

Stamm Test. - This test depends upon reaction of oxidized fat with s-diphenyl carbazide to yield a color.[185] It has been correlated with organoleptic detection of rancidity and found to give a rather good correlation.[186]

Dicarbonyl Compounds. - These compounds are supposedly present among the products of oxidation of fats, and O'Daniel and Parsons[187] have postulated

that the color developed by oxidized fats in alkali is due to the formation of quinones from α-dicarbonyl compounds by double aldol condensation. They suggested that alkali color is a test for α-dicarbonyls. Prill has also developed a color test involving their dioximes.[188] The alkali color test has been shown to be affected by fat concentration and by time.[186] Nevertheless, the α-dicarbonyl test was considered the best method for assessing rancidity because the dicarbonyl value of oils is not affected by heat treatments of the oils.

Alkali Color. - When oxidized fats are dissolved in an alkaline medium, intense orange or red colors are produced. This is the result of production of chromophores whose principal absorption is in the ultraviolet range. The color is merely absorption in the visible region by ultraviolet chromophores. The alkali color has been studied spectrophotometrically by Holman, Lundberg and Burr[189] and it was concluded that the color was probably not due to quinones formed from dicarbonyl in the oxidized fat. Jasperson, Jones and Lord[190] also concluded that dicarbonyl tests on oxidized fats are fundamentally different from those on model compounds. Present opinion is that the color is due to condensation of unsaturated carbonyl compounds. Chipault and Lundberg[191] have found that the alkali color is due to two reactions, one which is instantaneous, and one which continues over long periods.

Hendrickson, Cox, and Konen[192] used spectral study of the alkali color in assessing the oxidative changes taking place in film formation. Chipault and Lundberg[99,191,193] likewise used this color formation in describing the chemical changes during film formation from pure triglycerides, pentaerythritol esters, and alkyds prepared from known pure fatty acids.

It would appear that the α-dicarbonyl color reactions, based upon sketchy knowledge, however, do possess merit for assessing oxidation of fats. It is

to be hoped that a better understanding of their chemistry will lead to more effective use.

Ultraviolet Absorption. - Oxidation of polyunsaturated fatty acids is accompanied by increased ultraviolet absorption. The magnitude of change is not easily related to degree of oxidation because the effects upon the various unsaturated acids are different in quality and magnitude.[194] However, the spectral change for a given substance can be used as a relative measure of oxidation, and probably has its best application in the detection of oxidation rather than its measurement. The examination of ultraviolet spectrum is a rapid and sure method for assessing the purity and freshness of unsaturated fatty materials. The higher the absorption, the greater has been the exposure to oxygen.

Infrared Analysis. - Henick[195] has applied infrared analysis to the detection of oxidation products in milk fat. Spectral changes were detected before off flavors developed, and both loss of flavor and development of off flavors were correlated with definite absorption bands. Dugan, Beadle and Henick[195a] studied autoxidized methyl linoleate in the hydroxyl and carbonyl regions. They assigned the 3430-3445 cm.$^{-1}$ region to the -OOH group. Honn, Bezman and Daubert[195b] and Crecelius, Kagarise and Alexander[195c] employed infrared to follow the autoxidation of linseed oil. Study of the fats is extremely complicated spectrally.

Classification of Oxidation Reactions on a Temperature Basis. - The effect of temperature on the oxidation mechanism and the products of oxidation of unsaturated fats or fatty acids is such that for practical purposes three temperature ranges are readily distinguished and many investigations have been confined to one or another of these ranges. These temperature ranges are generally:

(1) atmospheric temperature or 0-40°C. (32-104°F.); (2) 60-120°C. (140-248°F.); (3) 200-300°C. (392-572°F.). Many of the oxidation reactions involved in the rancidification of fats occur at the lowest temperature mentioned. Blown oils, used in the drying oil industry, are generally prepared at temperatures between 60° and 120°C., and it is in this temperature range that most of the investigations on accelerated fat deterioration are conducted. The highest temperatures are employed in the manufacture of boiled oils, stand oils, and related polymerized fat products.

Atherton and Hilditch[196] measured changes in iodine value in methyl oleate and concluded that at 20° oxygen reacted with the methylenic group to form hydroperoxides, and also with the olefinic linkage. At 120° the reaction appeared to occur exclusively at the olefinic linkage and to be followed by secondary reactions at other points. Paquot[197] concluded that at 20° hydroperoxide formation predominated while at 120° a moloxide was formed.

From a study of the oxidation of elaidic acid and its esters, Skellon and Thruston[198] concluded that at 55° and 85° the oxidation reaction is marked by an induction period and that chemical changes at these temperatures are slight. At 120° the induction period is said to be absent and carbonyl compounds, carbon dioxide and polymers are formed.

The oxidation products of triglycerides have also been classified in this manner. In a recent paper[199] the products of the oxidation of fatty oils are reported to fall into four groups that pass into each other with considerable overlapping. The author classified the products on the basis of temperature and the products of the oxidation at any temperature are said to be a mixture of all types.

A difference in chemical nature among the oxidation products of lard formed at different temperatures has been demonstrated by Lewis and Quackenbush[57]

by the use of polarographic analysis.

The effects that differences in reaction temperature exert on the induction period and oxypolymerization of raw linseed oil have been investigated by Hess and O'Hara.[200] They found three distinct temperature regions, each characterized by different types of oxidative changes. The regions are: above 130°, between 84 and 130°, and below 84°. Temperature was found to have an effect on the induction period and on the peroxide values of the oil. Ultraviolet absorption analyses indicated that the formation of conjugated diene systems reached a maximum and that at temperatures between 84° and 200° the diene configuration was never appreciably greater than 5 per cent. Higher values were obtained at lower temperatures. In addition, these investigators proposed a chelate-type of intermediate in autoxidatively produced polymerization.

This classification of oxidation reactions on the basis of the temperature at which they take place should be of interest to all concerned with the processing of fats and oils.

Ricinoleic Acid and Methyl Ricinoleate. - Ellis[201] obtained unsaturated ketones from the autoxidation products of ricinoleic acid and showed that there is considerable polymerization at high temperatures. Autoxidation of methyl ricinoleate[10] with uranyl ricinoleate as catalyst gave typical oxygen absorption curves at 55°, 85° and 120°. At 85° rapid peroxidation occurred with little change in iodine number, suggesting addition of oxygen to the molecule without affecting the unsaturation. At later stages, the peroxide and iodine numbers decreased together.

Erucic and Brassidic Acids. - Skellon and Taylor[202] have recently described the autoxidation of erucic acid and of methyl and propyl erucates at 55°, 85° and 120°. Changes in the observed active oxygen content followed the usual

course, but the percentage of active oxygen was highest in the autoxidized propyl ester. The influence of alkyl groups was marked. The oxidation products consisted of small percentages of epoxides and cleavage products, ketohydroxy, dihydroxy, and aldehydo derivatives, together with oily complex products. Unsaturated keto derivatives were not conclusively identified.

The autoxidation of brassidic acid at 73° and 120° has also been studied.[203] In contrast to erucic acid, monoperoxy acids were not isolated from the autoxidation products. After saponification, dihydroxybehenic acid (8%) and an oily monomer (72%) were recovered. The latter may be either acyloin or an unsaturated ketonic derivative.

These results are qualitatively similar to those reported earlier by Dorée and Pepper,[204] who employed cobalt erucate as catalyst in most of their work. Yields of epoxides were as high as 15%.

10-Hendecenoic Acid. - The autoxidation of 10-hendecenoic acid or its methyl ester at 80° yields some sebacic acid, 10,11-dihydroxyhendecanoic acid and polymer.[205] The polymer may arise from aldol condensation of oxidation products containing carbonyl groups.

REFERENCES

1. L. Bateman, Quarterly Revs. 8, 147-167 (1954).

2. J. L. Bolland, Quarterly Revs. 3, 1-21 (1949).

3. W. Franke, Farben, Lacke, Anstrichstoffe 4, 301-311 (1950).

4. R. T. Holman, in "Progress in the Chemistry of Fats and Other Lipids", Pergamon Press Ltd., London, Vol. 2, Chapt. 2 (1954).

5. W. O. Lundberg, J. R. Chipault, and M. J. Hendrickson, J. Am. Oil Chemists' Soc. 26, 109-115 (1949).

6. S. G. Morris, J. Ag. Food Chem. 2, 126-132 (1954).

7. L. A. O'Neill, Chem. and Ind. 1954, 384-387.

8. C. Paquot, Oléagineux 2, 15-19 (1947).

9. R. P. A. Sims, Can. Chem. Proc. Inds. 35, 125-129,133 (1951).

10. J. H. Skellon, Chem. and Ind. 1951, 629-632; ibid. 1953, 1047-1049.

11. D. Swern, J. T. Scanlan, and H. B. Knight, J. Am. Oil Chemists' Soc. 25, 193-200 (1948).

12. D. Swern and J. E. Coleman, J. Am. Oil Chemists' Soc. 32, 700-703 (1955).

13. K. Täufel and H. Rothe, Angew. Chem. 61, 84-89 (1949).

14. T. de Saussure, Ann. chim. et phys. [27, 13, 337-362 (1820); ibid. 49, 225-240 (1832).

15. M. S. Cloez, Bull. soc. chim. [27, 3, 41-49 (1865).

16. G. J. Mulder, "Die Chemie der Austrocknenden Öle," Published by J. Springer, Berlin (1865). (In University of Pennsylvania Library.)

17. C. F. Schönbein, J. prakt. Chem. 74, 328-340 (1858); ibid. 75, 73-78 (1858).

18. A. Bach, Compt. rend. 124, 951-954 (1897); J. Russ. Phys. Chem. Soc. 29, 373 (1897).

19. C. Engler and J. Weissberg, Ber. 31, 3046-3055, 3055-3059 (1898). See also "Kritische Studien über die Vorgange der Autoxydation," Vieweg, Braunschweig (1904).

20. C. Engler and W. Wild, Ber. 30, 1669-1681 (1897).

21. C. Engler, Ber. 33, 1090-1096, 1097-1109, 1109-1111 (1900).

22. W. Fahrion, Z. angew. Chem. 22, 2083-2097, 2135-2144, 2187-2194 (1909); Chem. Ztg. 28, 1196-1200 (1904).

23. G. W. Ellis, J. Soc. Chem. Ind. 44, 401-408T, 463-468T, 469-472T, 486T (1925); ibid. 45, 193-199T (1926); Biochem. J. 26, 791-800 (1932).

24. H. Staudinger, Ber. 58, 1075-1079 (1925).

25. S. Fokin, Z. angew. Chem. 22, 1451-1459, 1492-1502 (1909).

26. D. Swern, Chem. Reviews 45, 1-68 (1949).

27. D. Swern, in "Organic Reactions", John Wiley & Sons, Inc. N. Y. Volume VII, Chapt. 7 (1953).

28. G. W. Ellis, Biochem. J. 30, 753-761 (1936).

29. H. N. Stephens, J. Am. Chem. Soc. 50, 568-571 (1928).

30. R. Criegee, Ann. 522, 75-96 (1936).

31. R. Criegee, H. Pilz and H. Flygare, Ber. 72, 1799-1804 (1939).

32. H. Hock and W. Susemihl, Ber. 66, 61-68 (1933).

33. H. Hock and O. Schrader, Naturwissenschaften 24, 159 (1936).

34. H. Hock, Oel, Kohle Erdoel Teer, 13, 697-700 (1937).

35. H. Hock and K. Gänicke, Ber. 71, 1430-1437 (1938).

36. E. H. Farmer and A. Sundralingam, J. Chem. Soc. 1942, 121-139.

37. A. Rieche, "Die Bedeutung der organischen Peroxyde für die chemische Wissenschaft und Technik," Enke, Stuttgart (1936).

38. A. Rieche, Angew. Chem. 50, 520-524 (1937).

39. E. H. Farmer, Trans. Faraday Soc. 38, 340-348 (1942); ibid. 42, 228-236 (1946); Rubber Chem. Tech. 19, 267-276 (1946).

40. E. H. Farmer, G. F. Bloomfield, A. Sundralingam, and D. A. Sutton, Trans. Faraday Soc. 38, 348-356 (1942).

41. E. H. Farmer and D. A. Sutton, J. Chem. Soc. 1943, 119-122, 122-125.

42. E. H. Farmer, Trans. Faraday Soc. 38, 356-361 (1942).

43. E. H. Farmer, H. P. Koch, and D. A. Sutton, J. Chem. Soc. 1943, 541-547.

44. A. J. Stirton, J. Turer, and R. W. Riemenschneider, Oil and Soap 22, 81-83 (1945).

45. F. D. Gunstone and T. P. Hilditch J. Chem. Soc. 1945, 836-841.

46. D. A. Sutton, J. Chem. Soc. 1944, 242-3.

47. C. E. Swift, F. G. Dollear, and R. T. O'Connor, Oil and Soap 23, 355-359 (1946).

48. J. Fugger, K. T. Zilch, J. A. Cannon, and H. J. Dutton, J. Am. Chem. Soc. 73, 2861-2864 (1951).

49. O. S. Privett, W. O. Lundberg, and C. Nickell, J. Am. Oil Chemists' Soc. 30, 17-21 (1953).

50. K. T. Zilch and H. J. Dutton, Anal. Chem. 23, 775-778 (1951).

51. J. E. Coleman, H. B. Knight, and D. Swern, J. Am. Chem. Soc. 74, 4886-4889 (195[illegible]

52. J. Ross, A. I. Gebhart, and J. F. Gerecht, J. Am. Chem. Soc. 71, 282-286 (1949).

53. E. H. Farmer and D. A. Sutton, J. Chem. Soc. 1946, 10-13.

54. H. B. Knight, C. R. Eddy, and D. Swern, J. Am. Oil Chemists' Soc. 28, 188-192 (1951).

55. C. O. Willits, C. Ricciuti, H. B. Knight, and D. Swern, Anal. Chem. 24, 785-790 (1952).

56. D. Swern, J. E. Coleman, H. B. Knight, C. Ricciuti, C. O. Willits, and C. R. Eddy, J. Am. Chem. Soc. 75, 3135-3137 (1953).

57. W. R. Lewis and F. W. Quackenbush, J. Am. Oil Chemists' Soc. 26, 53-57 (1949).

58. W. R. Lewis, F. W. Quackenbush, and T. de Vries, Anal. Chem. 21, 762-765 (1949).

59. H. Nogami, N. Matsuda, and K. Nagasawa, J. Pharm. Soc. Japan 71, 818-821 (1951).

60. C. Paquot and J. Mercier, Compt. rend. 236, 1802-1804 (1953); J. recherches centre matl. recherche sci., Labs. Bellevue (Paris) #24, 113-119 (1953).

61. C. O. Willits, C. Ricciuti, C. L. Ogg, S. G. Morris, and R. W. Riemenschneider, J. Am. Oil Chemists' Soc. 30, 420-423 (1953).

62. D. H. Saunders, C. Ricciuti, and D. Swern, J. Am. Oil Chemists' Soc. 32, 79-83 (1955).

63. G. King, J. Chem. Soc. 1954, 2114-2122.

64. E. H. Farmer, Trans. Inst. Rubber Ind. 21, 122-132 (1945).

65. J. L. Bolland and G. Gee, Trans. Faraday Soc. 42, 236-243, 244-252 (1946).

66. F. D. Gunstone and T. P. Hilditch, J. Chem. Soc. 1946, 1022-1025.

67. E. H. Farmer and D. A. Sutton, J. Chem. Soc. 1942, 139-148.

68. R. T. Holman, W. O. Lundberg, W. M. Lauer, and G. O. Burr, J. Am. Chem. Soc. 67, 1285-1292 (1945).

69. S. Bergström, Nature 156, 717-718 (1945); Arkiv Kemi, Mineral. Geol. 21A, #14, 1-18 (1945); ibid. #15, 1-8 (1945).

70. J. L. Bolland, Proc. Royal Soc. 186A, 218-236 (1946)

71. J. L. Bolland and W. J. C. Orr, Trans. Inst. Rubber Ind. 21, 133-138 (1945).

72. N. A. Khan, W. O. Lundberg, and R. T. Holman, J. Am. Chem. Soc. 76, 1779-1784 (1954).

72a. H. H. Sephton and D. A. Sutton, Chem. and Ind. 1953, 667.

73. D. H. Wheeler, in "Progress in the Chemistry of Fats and Other Lipids," Pergamon Press Ltd., London, Vol. 2, Chapt. 6 (1954).

74. J. E. Jackson, R. F. Paschke, W. Tolberg, H. M. Boyd, and D. H. Wheeler, J. Am. Oil Chemists' Soc. 29, 229-234 (1952).

75. P. L. Nichols, Jr., S. F. Herb, and R. W. Riemenschneider, J. Am. Chem. Soc. 73, 247-252 (1951).

76. O. S. Privett, W. O. Lundberg, N. A. Khan, W. E. Tolberg, and D. H. Wheeler, J. Am. Oil Chemists' Soc. 30, 61-66 (1953).

77. J. A. Cannon, K. T. Zilch, S. C. Burket, and H. J. Dutton, J. Am. Oil Chemists' Soc. 29, 447-452 (1952).

77a. P. L. Nichols, Jr., S. F. Herb, and R. W. Riemenschneider, J. Am. Chem. Soc. 73, 247-252 (1951).

78. W. O. Lundberg and J. R. Chipault, J. Am. Chem. Soc. 69, 833-836 (1947).

79. T. P. Hilditch, J. Oil Colour Chem. Assoc. 30, 1-16 (1947).

80. G. P. Gibson, J. Chem. Soc. 1948, 2275-2290.

81. O. S. Privett, C. Nickell, W. E. Tolberg, R. F. Paschke, D. H. Wheeler, and W. O. Lundberg, J. Am. Oil Chemists' Soc. 31, 23-27 (1954).

82. W. Kern, Fette u. Seifen, 53, 746-748 (1951).

83. W. Kern, A. R. Heinz, and J. Stallmann, Makromol. Chem. 16, 21-35 (1955).

84. W. Kern and A. R. Heinz, Makromol. Chem. 16, 81-88 (1955).

85. W. Kern, H. Jockusch, and A. Wolfram, Makromol. Chem. 3, 223-246 (1949).

86. W. Kern and J. Stallmann, Makromol. Chem. 7, 199-204 (1951).

87. H. Hock and M. Siebert, Chem. Ber. 87, 554-560 (1954).

88. R. S. Morrell, Chem. and Ind. 56, 795-798 (1937).

89. R. S. Morrell and E. O. Phillips, J. Soc. Chem. Ind. 58, 159-162 (1939).

90. A. B. Miller and E. Claxton, Ind. Eng. Chem. 20, 43-48 (1928).

91. R. W. Brauer and L. T. Steadman, J. Am. Chem. Soc. 66, 563-569 (1944).

92. R. T. Holman, W. O. Lundberg, and G. O. Burr, J. Am. Chem. Soc. 67, 1390-1394 (1945).

93. R. R. Allen, A. Jackson, and F. A. Kummerow, J. Am. Oil Chemists' Soc. 26, 395-399 (1949).

94. A. H. Jackson and F. A. Kummerow, J. Am. Oil Chemists' Soc. 26, 460-465 (1949).

95. J. E. Myers, J. P. Kass, and G. O. Burr, Oil and Soap 18, 107-109 (1941).

96. R. T. Holman and O. C. Elmer, J. Am. Oil Chemists' Soc. 24, 127-129 (1947).

97. R. R. Allen and F. A. Kummerow, J. Am. Oil Chemists' Soc. 28, 101-105 (1951).

98. H. P. Kaufmann, Fette u. Seifen 52, 140-143 (1950).

99. J. R. Chipault, E. C. Nickell, and W. O. Lundberg, Official Digest No. 322, 740-750 (1951).

100. H. H. Zuidema, Chem. Rev. 38, 197-226 (1946).

101. R. G. Larsen, R. E. Thorpe, and F. A. Armfield, Ind. Eng. Chem. 34, 183-193 (1942).

102. W. Langenbeck and W. Pritzkow, Fette u. Seifen 55, 506-511 (1953); Chem. Tech. (Berlin) 2, 116-118 (1950); ibid. 4, 391-393 (1952).

103. J. P. Wibaut and A. Strang, Proc. Koninkl. Nederland, Akad. Wetenschap. 54B, 102-109 (1951); ibid. 54B, 229-235 (1951); ibid. 55B, 207-218 (1952).

103a. G. H. Twigg, Chem. Eng. Sci. 3, Spec. Suppl., 5-16 (1954).

104. J. L. Benton and M. M. Wirth, Nature 171, 269 (1953).

104a. P. George, E. K. Rideal, and A. Robertson, Proc. Roy. Soc. 185A, 288-309 (1946).

105. F. de Goursac and C. Paquot, Oléagineux, 2, 564-567 (1947); C. Paquot and F. de Goursac, Compt. rend. 226, 258-260 (1948); Bull. soc. chim. France 1950, 172-173; Oléagineux 5, 349-363 (1950).

106. T. P. Hilditch, Nature, 166, 558-559 (1950).

107. J. L. Bolland, Trans. Faraday Soc. 44, 669-677 (1948).

107a. L. Kehren, Anais fac. farm. e odontol., Univ. Sao Paulo 10, 93-99 (1952).

108. N. A. Khan, J. B. Brown, and F. E. Deatherage, J. Am. Oil Chemists' Soc. 28 105-109 (1951).

109. F. E. Deatherage and H. A. Mattill, Ind. Eng. Chem. 31, 1425-1431 (1939).

110. J. L. Henderson and H. A. Young, J. Phys. Chem. 46, 670-684 (1942).

111. R. A. Max and F. E. Deatherage, J. Am. Oil Chemists' Soc. 28, 110-114 (1951).

112. N. A. Khan, J. Am. Oil Chemists' Soc. 30, 273-278 (1953).

113. J. H. Skellon, J. Soc. Chem. Ind. 50, 382-386T (1931).

113a. A. J. Feuell and J. H. Skellon, J. Chem. Soc. 1954, 3414-3418.

114. D. Swern, H. B. Knight, J. T. Scanlan, and W. C. Ault, J. Am. Chem. Soc. 67, 1132-1135 (1945).

115. H. B. Knight, E. F. Jordan, Jr., R. E. Koos, and D. Swern, J. Am. Oil Chemists' Soc. 31, 93-96 (1954).

116. J. E. Coleman, H. B. Knight, and D. Swern, J. Am. Oil Chemists' Soc. 32, 135-137 (1955).

117. J. E. Coleman and D. Swern, J. Am. Oil Chemists' Soc. 32, 221-224 (1955).

118. G. W. Ellis, Biochem. J. 46, 129-141 (1950).

119. G. W. Ellis, J. Chem. Soc. 1950, 9-12.

119a. J. H. Skellon and M. N. Thruston, J. Chem. Soc. 1949, 1626-1630; ibid. 1953, 138-142.

120. L. Williamson, J. Appl. Chem. (London) 3, 301-307 (1953).

120a. W. Kern, Farben, Lacke, Anstrichstoffe 4, 242-249 (1950).

121. C. E. Swift, F. G. Dollear, L. E. Brown, and R. T. O'Connor, J. Am. Oil Chemists' Soc. 25, 39-40 (1948).

122. C. E. Swift and F. G. Dollear, J. Am. Oil Chemists' Soc. 25, 52-53 (1948).

123. N. H. E. Ahlers and N. G. McTaggart, Analyst. 79, 70-76 (1954).

124. C. E. Swift, R. T. O'Connor, L. E. Brown, and F. G. Dollear, J. Am. Oil Chemists' Soc. 26, 297-300 (1949).

124a. O. C. Johnson, S. S. Chang and F. A. Kummerow, J. Am. Oil Chemists' Soc. 30, 317-320 (1953).

124b. F. K. Kawahara and H . J. Dutton, J. Am. Oil Chemists' Soc. 29, 372-377 (1952); F. K. Kawahara, H. J. Dutton and J. C. Cowan, ibid. 29, 633-635 (1952).

124c. R. J. Stopf and B. F. Daubert, J. Am. Oil Chemists' Soc. 27, 374-377 (1950).

124d. J. Brekke and G. Mackinney, J. Am. Oil Chemists' Soc. 27, 238-240 (1950).

124e. K. Meier and K. Ohm, Farbe u. Lack 59, 50-54 (1953).

124f. H. P. Kaufmann. and K. Strüber, Fette u. Seifen 54, 134-136 (1952).

124g. W. Kern and H. Willersinn, Makromol. Chem. 15, 15-35, 36-59 (1955).

125. A. Lund, Skrifter Norske Videnskaps-Akad. Oslo I. Mat.-Naturv. 1944, No. 3, 116p.

126. F. G. Smith and E. Stotz, N. Y. State Agri. Exp. Sta. Tech. Bull. No. 276 (1946).

127. J. H. Skellon, J. Chem. Soc. 1950, 2020-2023; J. H. Skellon and J. W. Spence, J. Soc. Chem. Ind. (London) 67, 365-368 (1948).

128. J. H. Baxendale, M. G. Evans, and G. S. Park, Trans. Faraday Soc. 42, 155-169 (1946).

129. A. Robertson and W. A. Waters, Trans. Faraday Soc. 42, 201-210 (1946).

129a. F. S. Greenawald, Off. Digest No. 281, 467-473 (1948).

129b. J. H. Skellon, J. Soc. Chem. Ind. 69, 116-120 (1950).

130. B. Andersson, Arkiv Kemi 2, 451-476 (1950).

130a. C. A. Klebsattel, J. Am. Oil Chemists' Soc. 27, 500-504 (1950).

130b. M. L. Kastens and F. R. Hansen, Ind. Eng. Chem. 41, 2080-2090 (1949).

131. P. George, Trans. Faraday Soc. 42, 210-216 (1946).

132. W. O. Lundberg, Hormel Inst., Univ. Minn., Pub. No. 20, 45 pp. (1947).

133. H. N. Alyea and H. L. J. Bäckström, J. Am. Chem. Soc. 51, 90-109 (1929).

134. J. L. Bolland and P. ten Have, Trans. Faraday Soc. 43, 201-210 (1947).

135. C. Golumbic, Oil and Soap 20, 105-107 (1943).

136. W. O. Lundberg, W. B. Dockstader, and H. O. Halvorson, J. Am. Oil Chemists' Soc. 24, 89-92 (1947).

137. H. S. Taylor, Biol. Antioxidants, Trans. 2nd Conf. 1947, 9-19.

138. L. Michaelis, Biol. Antioxidants, Trans. 3rd Conf. 1948, 11-23.

139. C. Golumbic, Biol. Antioxidants, Trans. 1st Conf. 1946, 42-48.

140. O. S. Privett and F. W. Quackenbush, J. Am. Oil Chemists' Soc. 31, 281-283 (1954).

140a. R. W. Riemenschneider, in "Handbook of Food and Agricultural," Academic Press, N. Y., Chapt. 8 (1955).

141. D. Barnard and K. R. Hargrave, Anal. Chim. Acta 5, 476-488 (1951).

142. C. Ricciuti, J. E. Coleman, and C. O. Willits, Anal. Chem. 27, 405-407 (1955).

143. C. H. Lea, Proc. Roy. Soc. 108 B, 175-189 (1931).

144. D. H. Wheeler, Oil and Soap 9, 89-97 (1932).

145. C. H. Lea, J. Soc. Chem. Ind. 65, 286-291 (1946).

145a. R. W. Riemenschneider, J. Turer, and R. M. Speck, Oil and Soap 20, 169-171 (1943).

146. W. O. Lundberg and J. R. Chipault, J. Am. Chem. Soc. 69, 833-836 (1947).

147. R. A. Chapman and W. D. McFarlane, Can. J. Res. B 21, 133-139 (1943); R. A. Chapman and K. Mackay, J. Am. Oil Chemists' Soc. 26, 360-363 (1949).

147a. H. Erdmann and F. Seelich, Z. anal. Chem. 128, 303-312 (1948).

148. C. H. Lea, J. Soc. Chem. Ind. 64, 106-109 (1945).

149. S. Hartmann and J. Glavind, Acta Chem. Scand. 3, 954-958 (1949).

150. L. Hartman and M. D. L. White, J. Sci. Food Agr. 3, 112-115 (1952).

151. J. Glavind, H. Granados, S. Hartmann, and H. Dam, Experientia 5, 84-85 (1949).

152. H. B. Knight and D. Swern, J. Am. Oil Chemists' Soc. 26, 366-370 (1949).

152a. H. B. Knight, J. E. Coleman, and D. Swern, J. Am. Oil Chemists' Soc. 28, 498-501 (1951).

152b. A. J. Fewell and J. H. Skellon, Analyst 78, 135-140 (1950).

153. R. T. Holman and N. A. Sørensen, Acta Chem. Scand. 4, 416-421 (1950).

154. F. J. Honn, I. I. Bezman, and B. F. Daubert, J. Am. Oil Chemists' Soc. 28, 129-133 (1951).

155. S. Bergström, R. Blomstrand, and S. Laurell, Acta Chem. Scand. 4, 245-250 (1950).

156. R. T. Holman and S. Bergström, in "The Enzymes," Academic Press, N. Y., Vol. 2, Part 1, Chapt. 60 (1951).

157. H. Schlenk, in "Progress in the Chemistry of Fats and Other Lipids," Pergamon Press Ltd., London, Vol. 2, Chapt. 5 (1954).

158. L. Bateman and G. Gee, Proc. Roy. Soc. A 195, 391-402 (1948).

159. N. A. Khan, W. E. Tolberg, D. H. Wheeler, and W. O. Lundberg, J. Am. Oil Chemists' Soc. 31, 460-466 (1954).

160. J. F. Mead, Science 115, 470-472 (1952).

161. S. Bergström and R. T. Holman, in "Advances in Enzymology", Interscience Pub., Inc., N. Y., 8, 425-457 (1948).

162. W. Franke, Ergeb. Enzymforsch. 12, 89-172 (1951).

162a. O. S. Privett, C. Nickell, W. O. Lundberg, and P. D. Boyer, J. Am. Oil Chemists' Soc. 32, 505-511 (1955).

163. A. L. Tappel, P. D. Boyer, and W. O. Lundberg, J. Biol. Chem. 199, 267-281 (1952); Arch. Biochem. Biophys. 42, 293-304 (1953).

163a. N. A. Khan, Arch. Biochem. Biophys. 44, 247-9 (1953).

163b. H. H. Strain, Acta Phytochim. (Japan) 15, 9-16 (1949).

164. G. O. Burr and R. H. Barnes, Physiol. Rev. 23, 265-278 (1943).

165. F. W. Quackenbush, Oil and Soap 22, 336-338 (1945).

166. R. H. Barnes, M. Clausen, I. I. Rusoff, H. T. Hanson, M. E. Swendseid, and G. O. Burr, Arch. sci. physiol. 2, 313-328 (1948).

167. P. L. Pavcek and G. M. Shull, J. Biol. Chem. 146, 351-355 (1942).

168. F. A. Kummerow, T. K. Chu, and P. Randolph, J. Nutr. 36, 523-536 (1948).

169. J. C. Fritz, J. L. Halpin, J. H. Hooper, and E. H. Kramke, Ind. Eng. Chem. 34, 979-982 (1942).

170. S. Lassen, E. K. Bacon, and H. J. Dunn, Arch. Biochem. 23, 1-7 (1949).

171. H. C. Stoerk, H. Kaunitz, and C. A. Slanetz, Arch. Path. 53, 15-21 (1952).

172. H. Kaunitz, R. E. Johnson, and C. A. Slanetz, J. Nutrition 46, 151-159 (1952).

173. S. M. Greenberg and A. C. Frazer, J. Nutrition 50, 421-440 (1953).

174. H. Kaunitz, C. A. Slanetz, and R. E. Johnson, J. Nutrition 55, 577-587 (1955).

175. P. W. Witten and R. T. Holman, Arch. Biochem. Biophys. 37, 90-98 (1952).

176. C. Widmer, Jr. and R. T. Holman, Arch. Biochem. 25, 1-12 (1950).

177. H. Kaunitz, C. A. Slanetz, R. E. Johnson, H. B. Knight, D. H. Saunders, and D. Swern, Federation Proc. 14, 408 (1955).

178. H. Kaunitz, C. A. Slanetz, R. E. Johnson, H. B. Knight, D. H. Saunders, and D. Swern, J. Am. Oil Chemists' Soc. 00, 0000 (0000).

178a. F. Bernheim, K. M. Wilbur, and C. B. Kenaston, Arch. Biochem. Biophys. 38, 177-184 (1952).

179. M. F. Pool and A. N. Prater, Oil and Soap 22, 215-216 (1945).

180. S. Patton, M. Keeney, and G. W. Kurtz, J. Am. Oil Chemists' Soc. 28, 391-393 (1951).

181. H. I. Kohn and M. Liversedge, J. Pharmacol. 82, 292-300 (1944).

182. F. Bernheim, M. L. C. Bernheim, and K. M. Wilbur, J. Biol. Chem. 174, 257-264 (1948).

183. K. M. Wilbur, F. Bernheim, and O. W. Shapiro, Arch. Biochem. 24, 305-313 (1949)

184. S. Patton and G. W. Kurtz, J. Dairy Sci. 34, 669-674 (1951).

184a. J. Glavind and S. Hartmann, Acta Chem. Scand. 5, 975-6 (1951).

185. J. Stamm, Analyst 51, 416-417 (1926).

186. G. A. Grant and H. J. Lips, Can. J. Res. F 24, 450-460 (1946).

187. L. O'Daniel and L. B. Parsons, Oil and Soap 20, 72-74 (1943).

188. E. A. Prill, Oil and Soap 19, 107-109 (1942).

189. R. T. Holman, W. O. Lundberg, and G. O. Burr, J. Am. Chem. Soc. 67, 1669-1674 (1945).

190. H. Jasperson, R. Jones, and J. W. Lord, J. Soc. Chem. Ind. 64, 143-145 (1945)

191. W. O. Lundberg and J. R. Chipault, J. Am. Chem. Soc. 69, 833-836 (1947).

192. M. J. Hendrickson, R. P. Cox, and J. C. Konen, J. Am. Oil Chemists' Soc. 25, 73-77 (1948).

193. J. R. Chipault, E. C. Nickell, and W. O. Lundberg, Off. Digest No. 328, 319-328 (1952).

194. R. T. Holman and G. O. Burr, J. Am. Chem. Soc. 68, 562-566 (1946).

195. A. S. Henick, Food Technol. 5, 145-147 (1951).

195a. L. R. Dugan, B. W. Beadle, and A. S. Henick, J. Am. Oil Chemists' Soc. 26, 681-685 (1949).

195b. F. J. Honn, I. I. Bezman, and B. F. Daubert, J. Am. Chem. Soc. 71, 812-816 (1949).

195c. S. B. Crecelius, R. E. Kagarise, and A. L. Alexander, Ind. Eng. Chem. 47, 1643-1649 (1955).

196. D. Atherton and T. P. Hilditch, J. Chem. Soc. 1944, 105-108.

197. C. Paquot, Oléagineux, 2, 15-19 (1947).

198. J. H. Skellon and M. N. Thruston, J. Chem. Soc. 1949, 1626-1630.

199. N. W. Gillam, Australian Chem. Inst. J. & Proc. 16, 19-36 (1949).

200. P. S. Hess and G. A. O'Hare, Ind. Eng. Chem. 42, 1424-1431 (1950).

201. G. W. Ellis, J. Chem. Soc. 1950, 9-12.

202. J. H. Skellon and P. E. Taylor, J. Chem. Soc. 1952, 1813-1816.

203. J. H. Skellon and C. G. Taylor, J. Chem. Soc. 1953, 1433-1435.

204. C. Dorée and A. C. Pepper, J. Chem. Soc. 1942, 477-483.

205. S. DalNogare and C. E. Bricker, J. Org. Chem. 15, 1299-1308 (1950).